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MORPHOGENESIS OF SOME FUNGI IN SUBMERGED CULTURES*

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Though our knowledge of mold growth in submerged fermentations extends as far back as 50 years, vast amount of data have accumulated in recent years following the starting up of antibiotic industry. With the exception of chloramphenicol, most of the antibiotics commercially produced have been obtained by submerged culture of micro-organisms. This has involved great amount of morphological studies of mycelial types with reference to antibiotic production. In the present account I wish to consider the morphogenesis of some fungi in submerged cultures which would indicate the need for more studies in this very interesting but little understood field.

Submerged growth is differentiated from surface growth cultures by the fact that the mold grows beneath the liquid surface provided proper aeration is available for the developing mold. Many of the fungi fail to grow in submerged cultures or fail to produce the valuable metabolic product such as antibiotics, vitamins, organic acids, etc., for which they are grown. From the point of view of chemical industry, submerged fermentations are more economical than surface cultures because large fermentor tanks can be made use of for sterile handling of the process, conserving space, equipment and time.

Historical

Since the main feature of obtaining successful submerged growth depends upon providing effective aeration, two processes are generally known. Submerged growth may be obtained either by aeration only, or aeration accompanied by mechanical agitation to provide more gaseous exchange. The effects of agitation on the morphylogy of the fungus was studied by Russell (1892) who observed bud-like growths rather than filamentous mycelium in some of the micro-organisms. Then, Ray in 1897 studied the effects of agitation on *Aspergillus candidus* and found that the growth rate in submerged culture was higher than that of still cultures and that it resulted in the formation of spherical pseudoparenchymatous structures. Sartory (1908) studied effects of agitation on species of Mucorales, Ascomycetes and Fungi Imperfecti and made microscopic studies of the mycelial morphology. He noticed that increase in agitation accentuated malformation of hyphae with the fragmentation of the colonies. The formation of spherical colonies was shown to be not universal.

Submerged mold fermentations on an industrial scale have been used for gallic acid by Calmette (1902), gluconic acid by Moyer et al (1940),

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lactic acid by Ward et al (1938) and adapted to penicillin fermentation in 1946. Study of growth in shake cultures adopting the shaking machine evolved by Kluyver and Perquin in 1933, has given great impetus for investigations on antibiotics, mold metabolism and morphology of the fungus. Large number of fungi may be studied at one time on a statistical basis under uniform controlled conditions. Pasteur (1876) was almost the first to aerate mold cultures artificially and study growth behaviour of *Aspergillus glaucus* and *Mucor racemosus*. He noted striking stimulation of growth of mycelium as a result of aeration. In the absence of aeration, however, large number of budding cells were formed which were comparable to the 'yeast phase' which could bring about fermentation of sugars.

While these and several other studies on morphology of the molds have been made by previous investigators, large-scale studies have been initiated only after the starting of the antibiotic industry. Considerable number of research work pertain to penicillin industry since it was the first antibiotic to be manufactured on an industrial scale and still holds the chief place among the group of antibiotics therapeutically used at present.

I. Type of mycelial formation in submerged cultures of *Pen. chrysogenum*.

Pellet formation: Since the starting of penicillin fermentation industry, the type of mycelial formation as affecting the penicillin production has been given special attention. Generally, two types of mycelia were recognised, one of them termed as pellets and the other long mycelium composed of branching filaments. The type of pellet formation is influenced by number of factors some of which we shall consider. Whether a pellet type of mycelium or a long mycelial type yields more antibiotic is a much debated point, since both the types are correlated often with high yielding fermentations. It may be said however, that in a small pellet the number of actively growing hyphae is more than in long mycelium, where the actively growing surface is restricted to the tips of branched hyphae. From an industrial point of view, pellety type of mycelium offers more difficulty in filtration on a rotary drum type of filter than the matty type of mycelium.

While the pellets have been observed by workers as early as 1908, their method of formation and factors influencing their formation have not been properly understood. As recently as 1949, Foster refers to their formation as aggregates of germinating spores combining with fragments of hyphae and often organising around foreign particles. Detailed studies on the morphogenesis of mycelial types in submerged cultures were initiated by Prof. Dr. E. B. Chain at the Institute Superiore di Sanita, Rome, and this has resulted in better understanding of the problem. A brief account of these is presented below.

(a) Factors influencing type of mycelium in shake flask cultures.

The formation of pellets or long mycelial type was investigated by Camici et al (1952). It was shown that heavy spore inoculum tended to

form pellets, but some of the other factors such as the age of spores, type of shaker etc. were not taken into account. These factors were studied by Thirumalachar and Gopalkrishnan (1956) who showed the following facts with reference to *P. chrysogenum*, strain Wis. 47-1956. Two spore concentrations (300 spores per ml. and 30,000 spores per ml.), 3 grades of spores (spores 10 days, 25 days and 40 days old) were used, using rotary shaker (220 r.p.m.) and reciprocating shaker (90 strokes per min). In 10 days' old spores pellets are formed, and high spore concentration and rotary shaker tend to increase long mycelial type of growth. The rotary shaker influences filamentous mycelial formation more readily than the reciprocating shaker. At low spore concentrations only pellets are formed on both rotary and receiprocating shaker. In old spores (40 days old) at high spore concentration few big pellets are formed on both rotary and reciprocating shakers while at low spore concentration there is only scanty growth. This is due to low percentage of spore germination. In case of 25 days old spores the following interesting feature has been noticed. At low spore concentration, only pellets are formed on both the types of shakers. With high spore concentration, only mycelium is formed on rotary shaker, while in the reciprocating shaker, both mycelium and pellets are formed. It was also evident that in those cases where pellets are formed their size and shape depended upon the initial spore inoculum. In some cases with 3 spores per ml. as inoculum, pellets up to 1 cm. in diameter have been obtained in the shake flasks.

Further studies by the writer on the pellet formation have revealed that the spores on germination develop germ tubes at either ends. These become septate and develop secondary branches similar to that described by Duckworth et al (1949) and Burkholder et al (1945). In those cases where a pellet is formed, the branches are developed in large numbers from the main germ tube. These branches radiate in all directions and due to close septation a central core of plectenchyma-like structure is formed, As more cells are added up, the central core increases in thickness, with the ends of the radiating hyphae projecting out. In those cases where the pellets are big, the central core of cells disintegrate due to autolysis and form a central cavity. This has been noticed by Camici et al (1952) also. From a primary pellet, the secondary pellets are formed by the incurling of strands of hyphae and the differentiation of another central core of cells. It has been noticed in particular that when a pellety type of growth is used as vegetative inoculum, it always results in pellety type of mycelium again and, even with any change in physical conditions like agitation, amount of inoculum, etc., does not revert to mycelial condition.

(b) Factors influencing type of mycelium in fermentors.

The results obtained from shake flask cultures may not always be borne out in the fermentors as the type of mycelial formation in a fermentor is influenced by several factors. The amount of aeration and agitation greatly affects the type of growth. The type of mycelial formation in a fermentor often gives a better picture of fermentation than most of the chemical analysis. Closely septate stout mycelium, occurrence of chlamydospores and swollen cells, autolysed hyphae etc. are often good indicators of poor formentation conditions. In a 10 litre fermentor using a stainless

steel sparger for aeration (where the bubbles formed are numerous and extremely small), Thirumalachar and Gopalkrishnan (1953) found the formation of sclerotic pellets. These had thick chlamydospore-like cells of resting nature, which when dried and stored, retained viability for long periods (over six months).

While the type of growth of a germinating spore is governed by the genetic character of the strain, it is partly influenced by cultural conditions also. Some of the strains never produce pellets, and others exclusively produce them under normal fermentation conditions. On the basis of morphological studies of the germinating spores conducted by the writer it has been possible to get some idea of the difference in the growth behaviour of the two types. The type of spore germination in the form which produces only pellets in the seed vessels is as follows. A stout germ tube is formed on one side, which becomes septate. By repeated dichotomous branching and close septation, a central core of cells is differentiated. Further development results in the formation of a pellet. Those strains which form a mycelial type of growth in seed vessels have spores germinating by germ tubes at two opposite poles. The secondary branches are long and attenuated and do not form a central core of cells. By further development a loose weft of mycelial growth is formed.

The rate of agitation of the propellors often modify the type of mycelial growth. In the long filamentous type of growth, the hyphae are fragmented due to mechanical damage caused by the blades. Following regeneration of the fragmented hyphae, clusters of branches are often formed comparable to witches' broom type of growth. In most cases the hyphal fragments remain as short mycelium.

Type of mycelial growth in submerged culture of some other groups of fungi:

In the course of studies on antibiotic substances and other metabolic products, several other fungi have been grown in submerged cultures, and peculiar features noted in some of them by the writer are presented here. *Romanoa terricola* Thirum. is a soil inhabiting ascomycete and member of the Clavicepitaceae. It produces cottony mycelium bearing numerous hyaline embedded perithecia. No conidial stages have been noticed in surface cultures in which it produces an antibacterial substance against Gram positive bacteria. In submerged cultures the mycelium at first makes an initial growth and then begins to fragment into numerous cells which begin to bud like yeast phase (Thirumalachar 1955). No antibiotic is produced in submerged culture.

The developmental morphology of three sclerotia producing fungi were studied by the writer in submerged cultures. *Sclerotinia sclerotiorum* forma *orobanches* Naras. & Thirum., *Corticium rolfsii* and *Botryodiplodia* (*Macrophomina*) *phaseoli* all produce abundant sclerotia on agar and liquid surface cultures. When grown in submerged culture of both synthetic and semi-synthetic media, only mycelial growth was observed without any indication of even rudiments of sclerotia in the first two forms. In contrast, *Botryodiplodia phaseoli* when grown in Czapek solution alone or with addition of 1% corn steep liquor, developed thick growth of mycelium in sub-

merged culture forming numerous sclerotia. In later stages the vegetative hyphae autolysed liberating free sclerotia with fragments of hyphae attached to them.

Conidial formation during submerged culture:

Sporulation of fungi in submerged growth is an interesting phenomena. Some of the fungi produce spores on short undifferentiated branches of hyphae, while others produce well-differentiated fruiting structure comparable to the normal type on agar surface cultures. *Volutellopsora cinnamomea* Thirum. produces sporodochium and astomous pycnidium in surface growth while in submerged cultures conidia are produced in large numbers on short branches. No sporodochia are formed. Several species of *Fusaria* behave in the same manner. Studies on *Colletorichum atramentarium* has also shown similar type of development. In *Aspergillus terreus* and in an antibiotic producing *Penicillium* species, the writer first found initial growth of mycelium followed by abundant sporulation. The different sources of carbon and nitrogen as also pH conditions had little effect in influencing the degree of sporulation. In *Aspergillus terreus*, from a small pellet, numerous conidiophores with well-developed foot-cells were formed in a radiating manner, and these produced typical vesicles, sterigmata and spores. Similar asymmetric divaricate type of penicilli were produced in the case of *Penicillium* species. Production of spores in submerged cultures of *Penicillium notatum* and *P. chrysogenum* were investigated by Foster et al (1945). Using sucrose-sodium nitrate medium and high concentration of calcium chloride (2.5 to 5%) abundant sporulation was obtained. The mycelial development was very sparse. Gilbert and Hickey (1946) noted that high calcium chloride concentration was not necessary to obtain high degree of sporulation, and using 25% grain stillage and 3% glucose in the media obtained abundant sporulation in submerged culture. It is evident that sporulation in submerged cultures is associated with poor conditions for vegetative growth. High concentration of antimetabolites, toxic substances, low pH etc. are some of the factors that induce sporulation in an otherwise non-sporulating culture.

The production of antibiotics, organic acids etc. by the molds is chiefly from the actively growing mycelium. When the mycelium breaks down into yeast phase or produces conidia etc., the metabolic activity of the mycelium to produce the antibiotics etc. is reduced. This is well-illustrated in species of *Fusarium*, and *Cephalosporium* species which have been found to produce antibiotic substance in surface culture, but in submerged culture form thick mass of spores with scanty vegetative hyphae and no antibiotic substance. In this connection mention may be made of addition of sporulation inhibiting substances which promote development of mycelium without interfering with antibiotic production. In the study of an acid stable penicillin-like antibiotic produced by a *Penicillium* species, profuse sporulation in various type of media reduced antibiotic production. Addition of 0.1% phenyl acetamide or 0.1% phenyl acetic acid to the medium completely suppressed sporulation and resulted in abundant antibiotic production. For several other fungi, addition of this small quantity of phenyl acetamide or phenyl acetic acid has been found to promote more vegetative growth by suppressing sporulation.

Submerged culture studies have often given details of spore forms which are met with very rarely in surface cultures. A species of *Emericellopsis*, closely related to *E. mirabilis* isolated from soil in Poona, produces very rarely the *Cephalosporium* stage (also described in the type of the genus). This is the same fungus genus recently reported by Cain under the name *Saturnomyces*. In submerged cultures, the fungus produced abundant conidia forming a thick suspension.

Development of sexual phase in submerged cultures:

The possible development of sexual phase in submerged cultures is of extreme interest. The present discussion refers to only two cases in Ascomycetes. In *Eremothecium ashbyii* which produces the valuable vitamin riboflavin, the cells of the hyphae transform into asci and develop numerous ascospores. The asci formation is not suppressed by the addition of phenyl acetamide etc. At the end of the fermentation cycle the broth is found to be full of ascospores released by the bursting of asci.

The species of *Emericellopsis* referred to, produces dense white mycelium when grown in Czapek solution supplemented with 1% corn steep liquor. After initial growth, numerous cleistothecia are formed, which appear as black specks in the mycelium. When placed on a rotary shaker for 7 days and incubated at 24°C., most of the perithecia mature. Unlike the ones formed on agar surface cultures, the number of asci that ripen are 2 to 8 only and the perithecia are empty in many cases. The ascospores in asci that mature are normal with the characteristic ridge and appendages. As far as the writer is aware, there is no previous record of perithecial or cleistothecial fruiting body in submerged culture.

From the above brief account, one may visualise the importance of study of morphology of the molds in submerged cultures and the need for more studies in this new field. The data obtained would be of extreme importance in the field of industrial mycology, particularly antibiotic production.

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EFFECT OF SOME ANTIBIOTICS ON XANTHOMONAS MALVACEARUM (E. F. Smith) Dowson AND ON THE MICROFLORA OF COTTON SEED

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Blackarm disease of cotton, caused by *Xanthomonas malvacearum* (E. F. Smith) Dowson, is one of the serious diseases of the crop. The pathogen is known to be carried through the seed in the form of external contamination of the fuzz and to a certain extent as deep-seated internal infection (Wickens 1953). Tarr (1953) tested chloromycetin, streptomycin, and penicillin as dry seed dressing agents and obtained partial control of the disease. The effects of some antibiotics on the pathogen in *vitro* and on the microflora of cotton seed, when used as liquid steep, were studied and the results are reported here.

The antibiotics used in these studies are: (1) streptomycin sulphate, a pharmaceutical preparation with 740 I.U./mg, manufactured by Glaxo Laboratories Ltd., England, (2) Agristrep Type A, prepared by Merck & Co., Inc., U.S.A., (3) Agrimycin 100, containing 15 per cent streptomycin and 1.5 per cent oxytetracyclino (Terramycin), prepared by Pfizer & Co., Inc., U.S.A. (4) Phytomycin (liquid), prepared by E.R. Squibb & Son, N. J., U.S.A., (5) Mycothricin complex B, containing 400 U/mg, prepared by the author at the Institute of Microbiology, Rutgers University, N.J., U.S.A., and (6) Mycostatin (crude crystalline form), containing 2740 U/mg, prepared by E. R. Squibb & Son, N. J., U.S.A.

The *in vitro* effect of the antibiotics on bacteria was studied by the agar streak assay method of Waksman and Reilly (1945). A twenty-four hour culture of a fresh isolate of *X. malvacearum* grown on nutrient dextrose agar slant was suspended in sterile distilled water and streaked on agar media containing serial dilutions of the antibiotic. A culture of *Bacillus subtilis* Cohn emend. Prazmowski prepared in a similar manner was also streaked in the same plates for comparison. The plates were incubated for 24 hours at room temperature (24 to 28 °C) and the results recorded (Table I).

Table I. The effect of the antibiotics on the growth of *X. malvacearum* in nutrient dextrose agar

Antibiotic	Minimal inhibibory concentration : mcg/ml	
	X. malvacearum	*B. subtilis*
Streptomycin sulphate	1.0	0.25
Agristrep	2.5	1.0
*Phytomycin	1.0	0.25
Agrimycin	5.0	1.0
Mycothricin	5.0	1.0
Mycostatin	100.0	100.0

*The concentrations refer to the streptomycin base contained in Phytomycin,

All the preparations containing streptomycin as the main active agent, viz., streptomycin sulphate, Agristrep, Agrimycin, and Phytomycin and also Mycothricin are quite effective in checking the growth of *X. malvacearum* at very low concentrations. Mycostatin, which is mainly an antifungal antibiotic, is not effective on the bacterium even at 100 mcg/ml.

In order to study the effect of the antibiotics on the microflora of cotton seed, MCU 1, a variety of cotton known to be highly susceptible to blackarm disease, was selected. The seed was treated with various antibiotics at 500 and 1,000 p.p.m. in water for four hours, dried in the shade on sterile blotting papers and plated on nutrient dextrose agar, Twenty-five seeds from each treatment were examined and the number of bacterial and fungal colonies developing from them recorded after forty-eight hours' incubation at room temperature (Table II).

TABLE II. The influence of antibiotic treatment on the total number of bacterial and fungal colonies developing from cotton seed (25 seeds per treatment plated on nutrient dextrose agar)

Antibiotic	Concentration of the antibiotic			
	500 p.p.m.		1,000 p.p.m.	
	No. of bacterial colonies	No. of fungal colonies	No. of bacterial colonies	No. of fungal colonies
Streptomycin sulphate	0	18	0	21
Agristrep	0	25	0	15
Phytomycin	0	20	0	14
Agrimycin	0	22	0	16
Mycothricin	0	12	0	14
Mycostatin	25	0	14	0
*Agrosan GN	—	—	4*	6*
*Ceresan	—	—	2*	2*
Control (untreated)	20	19	16	24

*Both Agrosan GN and Ceresan were applied dry at the rate of 2 g/lb of seed.

The four streptomycin preparations and mycothricin exhibit selective inhibitory effect on the fungi at 1,000 p.p.m. Mycostatin is highly effective on fungi but not on bacteria. Agrosan GN and Ceresan seem to be equally effective on both the fungi and bacteria.

The colonies of bacteria and fungi developing from the seed were examined microscopically; the fungi consisted mainly of the common molds, *Rhizopus, Penicillium,* and *Aspergillus,* and the bacterial colonies mostly of the blackarm bacterium and some spore formers.

Tissues taken aseptically from inside the seeds treated with the streptomycin preparations and mycothricin were plated on nutrient dextrose agar impregnated with viable cells of A. *subtilis* and incubated at room temperature for twenty-four hours. Inhibition zones in the growth of the bacterium were observed around the tissues thereby indicating the presence of the antibiotic inside the tissues. No such inhibition was observed when tissues from untreated seeds were plated in the same manner. Furthermore, no bacterial colonies developed from the treated tissues while in the case of untreated tissues bacterial colonies were readily isolated. In a preliminary test it was found that none of the antibiotics exerted any inhibitory effect on the germination of the seed at the concentrations tested.

These results indicate that the antibiotics are active on the bacteria borne internally and externally on the cotton seed. They are also selectively active on the bacteria, leaving the fungal spores carried on the seed mostly unaffected. The beneficial effects of these fungi to the germinating seed are not inconsiderate especially in the light of the recent reports that certain organisms are capable of producing antibiotics on seed coats and other natural substrata (Wright 1956, Brain *et al* 1956). Streptomycin and mycothricin are known to be readily absorbed by plant tissues and transmitted systematically (Crowdy and Pramer 1955, Rangaswami 1956). Their presence in the seed tissues, therefore, is bound to be beneficial to the growing seedling which is susceptible to the bacterial infection. Studies on the phytotoxicity and systemic translocation of the antibiotics in the plants are in progress and the results will be published later.

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FUSARIUM WILT OF GARDEN PEAS (PISUM SATIVUM L.) IN BOMBAY STATE

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Introduction

Garden pea (*Pisum sativum* L.) occupies an area of about 14,557 acres in Poona and North Satara districts of Bombay State. In North Satara, it is usually grown in the *rabi* season, whereas its cultivation in the *kharif* season is restricted to Poona district. Though pea wilt had appared earlier in the State in a sporadic form, it had not assumed serious proportions till 1952 when it caused heavy losses in North Satara district. A detailed investigation of the disease was, therefore, undertaken and the results are recorded here.

Symptoms

The initial and most characteristic symptom of pea wilt is the recurving of the margins of the young stipules and leaflets, and rolling of leaves. Simultaneously, the upper parts of the plants may become pale, the growth of terminal bud is checked, the stem and upper leaves may become more rigid than normal and the roots more crisp and brittle, while the lower leaves turn pale and commence to wither. Sometimes the entire plant becomes yellow and the lower leaves wither progressively upwards. Characteristically, however, after the collapse of a few basal leaves, the upper part of the plant wilts abruptly and may become dry while still green in colour. After wilting, the stem shrivels downwards from the tip towards the basal internode, which remains firm and turigd till the end. Discolouration of the vascular system, partial wilting and sterility, so characteristic of *Fusarium* wilts, are noticed. If pods are formed, they contain only a few shrunken, immature seeds which soon dry up. The disease, as it occurs in Bombay State, resembles the *Fusarium* wilt of peas in the U.S.A.

Pathogenicity

Fusarium sp. was easily isolated in artificial culture from roots and stems of wilted plants. The pathogenicity of the fungus was proved by growing plants of peas (var. Mahableshwar) in steamed soil infested with a pure culture of the fungus grown on Richard's solution and stored for a month. This soil was filled in clay pots which after sowing of seeds were placed in a glass-house where the mean temperature fluctuated between 23 and 28°C. Adequate uninoculated controls were provided.

Wilt appeared 23 days after germination of seeds and progressed rapidly thereafter; all plants were killed in two months. Isolations from

wilted plants invariably yielded a species of *Fusarium* which was indentical with the culture with which the soil was infested.

Morphology and Cultural Characters

The fungus was grown on a variety of media to obtain a complete range of spores as recommended by Wollenweber *et al* (1925); synthetic agar, potato hard agar and Richard's agar were also used as suggested by Doidge (1939). The cultures were incubated at 24–27 °C. and observations made regularly. Ridgway's (1912) colour nomenclature was used. No sporodochia, pionnotes, or sclerotia were produced on any media. Microconidia and chlamydospores were produced on all media whereas pigment was evident on a few. The results are summarized in Table I.

Average measurements of conidia:—

0–Septate,	8.5 x 4.3	(4.2–17 x 2–5)	Microns
1– ,,	14.6 x 4.1	(9.0–20 x 2–5)	,,
2– ,,	19.6 x 4.0	(14–26 x 3–6)	,,
3– ,,	30.6 x 4.5	(25–35 x 3–5)	,,
4– ,,	33.5 x 4.1	(30–37 x 3–5)	,,
5– ,,	38.1 x 3.3	(36–42 x 2–4)	,,

Microconidia are mostly 0-septate (0–3), irregularly ellipsoidal to elongate, somewhat curved, borne on the mycelium either above or within the substratum, usually in abundance. Macroconidia rarely produced 3–5 septate, straight to slightly curved, hyaline, nonpedicellate and with delicate walls and septa. Chlamydospores abundant, mostly simple, rarely compound, thick-walled, tuberculate, both terminal and intercalary. Mycelium well-developed variable in diameter (2–4 μ) and septation, white at first, tawny to variously tinted in mass; pigmentation of substratum highly variable.

Physiology of the Fungus

Relation of temperature to fungus growth

The temperature growth relations of the fungus were studied on triplicate plates of potato dextrose carried at various temperatures and growth was measured as mean colony diameter after six day incubation. The results are recorded below.

Temperature-growth relations of the fungus

Temperature °C	Mean colony diameter after 6 days (cms).
5	0.0
8	0.3
10	0.6
15	2.7
25	7.0
27	8.0
30	5.5
32	4.2
35	0.5
40	0.0

TABLE I: Cultural characteristics and measurements of conidia and chlamydospores (in) of pea Fusarium on different media

Character	Oatmeal agar	Richards' agar	Synthetic agar	Potato dextrose agar (2% dextrose)	Potato dextrose agar 5(% dextrose)	Potato hard agar	Proteose peptone agar	Potato plug	Steamed rice	Pea stem
1	2	3	4	5	6	7	8	9	10	11
Growth	Thin	Profuse	Moderate	Profuse	Profuse	Scanty	Moderate	Sparse	Moderate	Sparse
Colour of aerial mycelium	Corythion red	White	White	Hortense violet	Hortense violet	White	Phlox purple	White	Crimson	White
Colour of substrate	Mauve	Light buff	Pale quaker drab	Contigna purple	Contigna purple	None	Vinaceous brown	None	Congo red	None
Sporulation										
Chlamydospores) measurement in microns)	—	Single or in pairs 13.3x10.2	Single 9.2 x 7.3	Single in pairs 12.2 x 9.5	Single or in pairs 12.4 x 9.4	—	Single 11.3 x 9.0	Single 9.4 x 7.1	Single or in pairs 12.3x10.0	Single 7.8 x 6.9
Comidis										
0—septate	44 percent 8.0 x 3.6	60 percent 11.2 x 4.6	86 percent 11.8 x 5.1	90 percent 9.6 x 4.1	64 percent 10.4 x 4.3	85 percent 8.7 x 4.4	88 percent 7.2 x 3.7	78 percent 10.5 x 4.7	88 percent 9.3 x 4.1	49 percent 8.6 x 4.5
1—septate	20 percent 14.5 x 3.7	26 percent 17.3 x 5.1	10 percent 16.2 x 5.1	10 percent 13.7 x 4.9	20 percent 14.9 x 5.2	15 percent 13.1 x 4.5	8 percent 12.9 x 4.5	10 percent 14.4 x 4.7	8 percent 13.2 x 5.0	26 percent 15.0 x 4.2
2—septate	10 percent 20.6 x 4.1	10 percent 22.9 x 5.6	4 percent 22.2 x 4.3	—	8 percent 20.2 x 5.0	—	4 percent 19.6 x 3.6	6 percent 20.8 x 4.6	6 percent 20.4 x 4.6	10 percent 20.3 x 3.9
3—septate	12 percent 30.4 x 4.6	2 percent 31.3 x 5.6	—	—	4 percent 27.8 x 6.4	—	—	4 percent 30.8 x 4.2	—	8 percent 32.0 x 3.4
4—septate	10 percent 33.0 x 4.0	2 percent 33.6 x 4.4	—	—	4 percent 30.2 x 4.5	—	—	2 percent 35.2 x 4.0	—	4 percent 37.8 x 3.4
5—septate	4 percent 36.2 x 3.6	—	—	—	—	—	—	—	—	3 percen 40.1 x 3.0

The fungus has a wide growth-temperature range, the optimum being 27 °C, and the minimum and maximum about 8 °C and 35 °C. respectively. These results are in agreement with those of Linford (1928) and Togashi (1931).

Utilization of Nitrogenous substance

The fungus when grown on Richard's medium (minus nitrate) to which different nitrogenous substances were added, could utilise a wide range of these sources, both organic and inorganic. It made best growth on Richard's agar containing potassium nitrate, commonium lactate and ammonium tartarate. Sodium nitrite was toxic to growth. Growth on media containing creatine, glutamic acid, glycocyamine and norvaline was poor. The fungus could utilise sodium nitrate, ammonium phosphate (dibasic), ammonium sulphate, ammonium nghrate, tryptophane, urea, asparagine, arginine and alanine to some extentand asparatic acid, tyrosine, peptone, methionine, glycine and norleucine slightly.

Utilization of Carbon compounds

Richard's solution (containing sucrose) was the best medium for fungus growth. Reffinose, dextrose and galactose were also utilised fairly well, but pectin, arabinose. xylose, salicin and lactose were not suitable for growth.

Hydrogen-ion in relation to growth

The fungus could grow at pH 5.3 to 9.7 but growth decreased in media with high acidity and alkalinity. The range of optimum reaction lies between 7.1 and 8.3 indicating that alkaline conditions are more favourable for the growth as reported by Walker and Snyder (1933).

Host Range

About 50 seeds of each of the following plants were sown in soil infested with the pea *Fusarium* kept in a glasshouse:—*Pisum sativum* L., *P. sativum* var. *arvense* Poir, *Lathyrus odoratus* L., *Lathyrus sativus* L., *Cicer arientinum* L., *Dolichos biflorus* Roxb., *Phaseolus radiatus* L., *P. aconitifolius* Jacq., *Cajanus Cajan* Millsp. and *Gossypium herbaceum* L.

After two months, plants were uprooted and the roots examined for discolouration. The results indicated that the fungus is restricted in its parasitism to *P. sativum* and *P. sativum* var. *arvense* only.

Varietal Resistance

Several varieties of peas from India, six resistant varieties from the University of Wisconsin, U.S.A. and five from Holland were tested for their resistance to the *Fusarium* under study. The results of this experiment are given in Table II.

TABLE II. VARIETAL RESISTANCE OF PEA VARIETIES TO FUSARIUM WILT

Name of variety	No plants tested	No. plants wilted	Percent wilt
Indian Varieties			
Mahableshwar (control)	200	200	100
Laxton's progress	20	20	100
American wonder	20	20	100
Early badger	10	9	90
Early Giant	10	9	90
Sugar Pills	20	16	80
Wrinkled seeded	20	14	70
Thomas Laxton	20	12	60
Upper India	20	12	60
Delwiche Commando	10	6	60
Bonneville	10	5	50
First to report	10	4	40
Phenomenon	10	4	40
American resistant varieties			
Resistant Alakha	20	16	80
New Era	20	12	60
Wisconsin Perfection	20	10	50
Wisconsin Early Sweet	10	5	50
Pride	20	8	40
Dutch resistant varieties			
Celsior	20	20	100
Alaska	20	18	90
Vares	20	18	90
Korza	20	16	80
Zelka	20	15	75

These results were confirmed by repeating the experiment. It was interesting to note that all the wilt resistant varieties from Wisconsin and Holland proved susceptible to the *Fusarium* under study.

REVIEW OF LITERATURE AND IDENTITY OF THE PATHOGEN

A large number of *Fusaria* have been isolated from wilted pea plants by various worker but only two are responsible for causing scrious wilt diseases on a mass scale. These cause the "Fasarium Wilt" and the "Near Wilt".

The "Fusarium Wilt" of peas was first recorded in Wisconsin in 1924–25 by Linford (1928) who named the causal fungus *Fusarium orthoceras* App. and Wr. var. *pisi* Linford, now named *F. oxysporum* Schlecht.

f. *pisi* (Lindford Synder and Hansen race 1) (Snyder and Hansen, 1940). The severity of the disease led to work on evolving wilt-resistant types, many of which are now in general cultivation in Northern United States. However, many of these wilt-resistant types were found to be affected by another Fusarium wilt resulting in the description of a second wilt disease of peas (Walker and Snyder, 1933), called "Near Wilt" on account of their similarities in symptoms. The "Near Wilt" pathogen, which closely resembles the "Fusarium Wilt" fungus, was named *F. oxysporum* Schlecht. f. 8 Snyder and Walker, now called *F. oxysporum* Schlecht. f. *pisi* (Linford) Snyder and Hansen race 2.

The cultural and morphological characters of the fungus under study and its spore measurements show that it closely resembles *Fusarium oxy sporum* Schlecht. f. *pisi* (Linford) Snyder and Hansen race 1. As regards pathogenicity, the fungus is restricted to *Pisum* spp. only. It differs, however, from the American pathogen in its ability to cause wilt in established commercial wilt-resistant American varieties of peas. It is thus evident that the fungus under study is distinct from the American one and it is, therefore, felt that the former should be designated a biotype of the American fungus, both the fungi resemble each other in all cultural and morphological characters but differ in pathogenicity to named wilt-resistant pea varietes.

Summary

Pea (*Pisum sativum*) L. suffers from a serious wilt disease in North Satara. Rolling and dropping of leaves is the first noticeable symptom and start from the oldest leaves. The whole plant than slowly dies and dries up. Isolations from wilted plants yielded a pathogenic species of *Fusarium,* whose morphological and cultural characters are described. The fungus has a growth temperature range of 8°–35°C., the optimum being 27°C. It is highly specialized since it does not infect plants other than peas. All wilt-resistant American and Dutch pea varieties proved susceptible.

The fungus belongs to subsection Orthocera in the Section Elegans of the genus *Fusarium* and resembles *Fusarium oxysporum* Schlecht. f. *pisi* (Linford) Snyder and Hansen, race 1, in its morphology and physiology but differs in its pathogenicity to named commercial wilt-resistant American and Dutch pea varieties. It is, therefore, proposed to name the Bombay fungus a biotype of the American pathogen.

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ALTERNARIA BLIGHT OF CLUSTERBEANS

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Introduction

Clusterbeans or guar (*Cyamopsis tetragonoloba* Taub.) is one of the important crops grown for vegetable, green manure or fodder in various parts of India. In 1918, Butler described a leaf spot disease of the crop caused by an organism identical to *Alternaria brassicae* (Berk.) Sacc. The disease was reported to be present in Pusa, Bihar State, and in Madras. In 1935, Narasimhan recorded an unidentified species of *Alternaria* on guar in Mysore. Uppal *et al* (1935) have listed. *A. brassicae* on *C. tetragonoloba* among the fungi of Bombay. An *Alternaria* leaf spot of *C. tetragonoloba* grown as a green manure crop in Arizona, U.S.A., was reported by Streets (1948). A similar leaf spot of the crop was recorded in Georgia by Luttrell (1951). He tentatively identified the organism as *A. cucumerina* (Ell. & Everh.) Elliot; none of the 55 strains of the crop introduced into the State from India showed any resistance to the disease. No detailed investigation of the disease, however, seems to have been made so far.

A severe *Alternaria* blight of the crop was observed during October-December 1953 in the Central Farm and Orchard, Agricultural College and Research Institute, Coimbatore. The disease was observed in various intensities in subsequent years in Coimbatore, Tanjore and other places. A detailed study of the disease was undertaken and the results are reported in this paper.

Symptoms of the Disease

The disease manifests mainly on the leaf blade as dark brown, round to irregular spots varying from 2 to 10 mm. in diameter. In the early stages of infection water-soaked spots appear on the leaf blade which later turn greyish to dark brown with concentric zonations, demarked with light brown lines, inside the spots (Fig. 1). On the under surface the spots are light to greyish brown. In severe infections several spots merge together involving a major portion of the leaf blade. In such cases the leaflet becomes chlorotic and usually drops off. In advanced stages of the disease the plants get severely defoliated and fail to put forth new flush. If the plants are affected in the early stages of growth, there may not be any flowering thereby causing severe economic loss to the cultivator, but if infected in advanced stages, the damange caused is very little, except for a few localized spots. Under favourable climatic conditions the disease may spread very quickly involving all the plants in a field.

Material and Methods

The fungus was isolated from the infected leaf-bits by the single-spore culture technique. Infection experiments were carried out by transferring to the leaf blade equal quantities of a pure culture of the fungus, grown on oat meal agar for 15 days. The inoculum was kept moist by covering with a bit of sterilized absorbant cotton wool, dipped in sterile distilled water. C.P. 78, a susceptible variety of clusterbeans, was used in all the infection experiments. On inoculation, the plants were covered with alkathene bags and high humidity was maintained by spraying water twice a day.

To study the host range of the organism various host plants were raised in the green-house and inoculations made when the plants were approximately one month old. Fresh isolates of *A. solani* (Ell. & Mart). Jones & Grout from tomato leaves, *A. brassica* (Berk.) Sacc. from radish leaves, and *A. brassicicola* (Schwein) Wiltshire from cabbage leaves were made and used in the studies to compare their morphology and host range with those of the clusterbean isolate.

The morphological characters of the fungus on the host and in agar media were studied and comparisons made with those of other related species. The cultural characters of the isolates were studied by growing them in Petri-dishes containing equal quantities of the various standard media; five mm. culture discs cut from actively growing regions of a five day culture of the fungus was used as inoculum; the dishes were incubated at room temperature (22 to 28°C.) and periodical observations made.

Experimental

1. *Pathogenicity:*

The pathogenicity of the fungus was tested by inoculating young leaves of clusterbeans in the manner described earlier. Plants of various ages, ranging from 15 days to three months, were used. Symptoms of infection could be seen within three days after inoculation and typical spots developed in five days. Several isolations of the fungus were made from specimens collected during 1953–56 and tested for pathogenicity. Successful infections were invariably obtained with all the isolates. The fungus was reisolated from the infected leaves and was found indentical with the original isolate.

2. *Host Range:*

In order to determine the host range of the fungus, some of the important vegetable crops of the locality and certain other hosts which are commonly affected by the leaf spot disease were inoculated with the fungus and incubated under optimum conditions. Cross inoculations with the isolates of *A. solani*, *A. brassica*, and *A. brassicicola* were also made in a similar manner. The symptoms of successful infection were observed in three to five days and typical spots produced in a week. The final results were recorded after ten days (Table 1).

TABLE I HOST RANGE OF *Alternaria* SP. FROM CLUSTERBEANS AS COMPARED TO THOSE OF RELATED SPECIES

Host	*A. solani*		*A. brassicae*		*A. brassicicola*		*Alternaria* sp. from clusterbeans	
	No. of leaves		No. of leaves		No. of leaves		No. of leaves	
	inoculated	infected	inoculated	infected	inoculated	infected	inoculated	infected
Solanum tuberosum L.	8	8	8	0	8	0	8	8
Cyamopsis tetragonoloba Taub.	8	5	8	0	8	0	8	8
Lycopersicon esculentum Mill.	8	8	8	0	8	0	8	4
Solanum melongena L.	8	8	8	0	8	2	8	2
Capsicum annuum L.	8	0	8	0	8	0	8	0
Datura stramonium L.	8	2	8	5	8	7	8	2
Raphanus sativus L.	8	5	8	5	8	8	8	8
Brassica juncea Hk. f. & T.	8	4	8	3	8	8	8	8
Gossypium hirsutum L.	8	0	8	0	8	0	8	0

The clusterbeans isolate readily infects potato, radish, and mustard, besides clusterbeans, but it is less infective to tomato, brinjal, and datura; it does not infect chillies and cotton. The host range of *A. solani* appears to be similar to that of the clusterbeans isolate, but it is more infective to brinjal and tomato and less so to clusterbeans, radish and mustard. *A. brassicae* and *A. brassicicola* have a similar but narrow host range as compared to that of the clusterbeans isolate; they do not pass on to clusterbeans, potato, tomato, brinjal, etc. Thus, it is evident that the clusterbeans isolate is distinct from the rest in its host range.

3. *Morphological Characters:*

The fungus produces abundant spores on the host. Both inter and intra-cellular hyphae are seen in transverse sections of the leaf passing through infected regions. Amphigenous conidiophores arise in groups of two to eight through the stomata. They are greyish olive in colour and geniculate with prominent scars at each geniculation (Fig. 2). Conidia are borne in straight chains of upto four, usually straight, obclavate, light greyish olive coloured, smooth surfaced, with depressions along the septa.

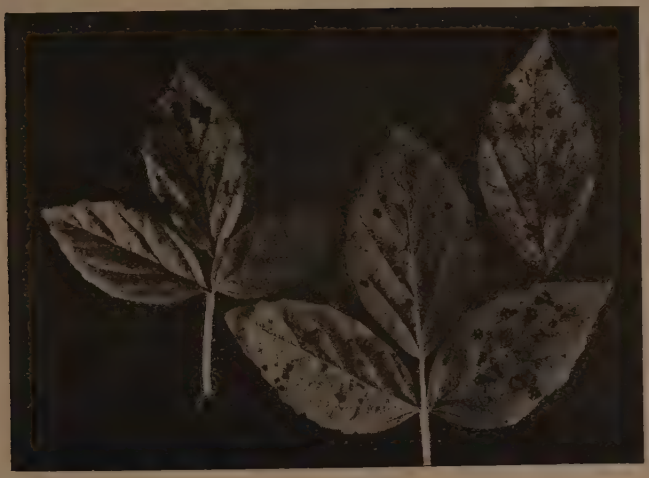

Fig. 1. Symptoms of Alternaria blight on the leaflets of clusterbeans.

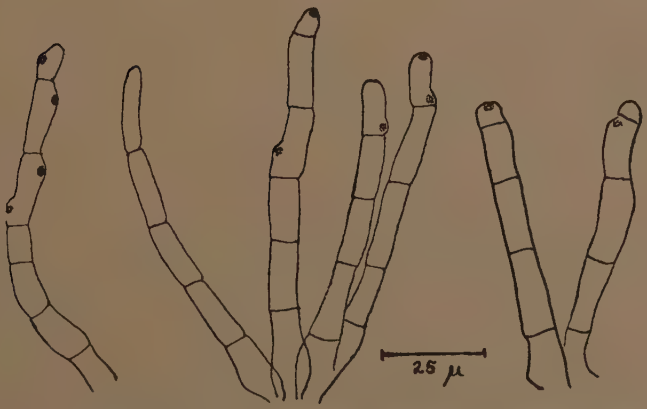

Fig. 2. Conidiphores of *Alternaria cyamopsidis* V. Rang & Rao

The beak is usually long varying from ½ to 1½ times the length of the spore, measuring 50 to 140μ and in general lighter coloured than the conidia. The conidia are muriform with 6 to 11 cross septa and 0 to 5 vertical or oblique septa. A comparison of the spore measurements of the various isolates is given in Table II.

TABLE II A COMPARISON OF THE SPORE MEASUREMENTS OF *Alternaria* SP. FROM CLUSTERBEANS WITH THOSE OF THE RELATED SPECIES

Species	Conidia		Conidiophore	
	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)
A. solani				
From host (potato)	102 — 322	12 — 28	40 — 121	5 — 9
According to Butler (1918)	145 — 370	16 — 18	50 — 90	8 — 9
A. brassicicola				
From host (cabbage)	19 — 118	6 — 22	37 — 76	3 — 6
According to Wiltshire (1947)	18 — 130	8 — 30	20 — 70	5 — 8
A. brassicae				
From host (radish)	40 — 112	9 — 19	56 — 99	3 — 6
According to Wiltshire (1947)	76 — 350	11 — 42	14 — 171	6 — 11
Alternaria sp. from clusterbeans –				
From host (clusterbeans)	62 — 146	12 — 19	47 — 78	3 — 6

4. *Cultural Characters:*

The isolate from clusterbeans grows well on oat meal, potato dextrose, Richards', Brown's, French bean, and clusterbean extract agar media. There was good growth of the fungus on potato dextrose agar adjusted to various pH, ranging from 5.0 to 9.0, pH 7.0 being the optimum. A comparison of the cultural characters of the various isolates was made by growing them on standard media under identical conditions. *A. solani* is distinct from the rest in producing a zinc-orange, soluble pigment in oat meal agar, potato dextrose agar, and other organic media. *Alternaria* sp. from clusterbeans, *A. brassicae*, and *A. brassicicola* did not produce any soluble pigment in any of the media tested.

A. brassicae produces an ivy green coloured mycelium on oat meal and potato dextrose agar media and in this respect closely resembles *A. brassicicola*. *Alternaria* sp. from clusterbeans, on the other hand,

produces quaker drab coloured mycelium on oat meal agar and dark purplish grey coloured mycelium on potato dextrose agar. Both *A. brassicae* and *A. brassicicola* produce abundant spores on all the media tested within three days, whereas the clusterbeans isolate produces spores in most of the media after an interval of 8 to 10 days. One of the isolates from Aduthurai, Tanjore District, however, failed to produce spores on any of the media tested. The spores from the clusterbean isolate grown on the agar media were measured and found to be comparatively smaller in size than those on the natural host. The beak also was less prominent being only 1/3 to 3/4 the length of the spore.

Discussion and Conclusion

Alternaria leafspot is one of the most common diseases of many cultivated and wild plants. Several species of the genus have so far been described and in some cases varieties of the species differentiated. The description of Butler (1918) that the *Alternaria* on clusterbeans is similar to that of *A. brassicae* on radish has been revised by Mason (1929), who after examining Butler's specimen identified the fungus as *A. circinans* (Berk. & Curt.) Bolle. *A. circinans* has subsequently been renamed *A. brassicicola* by Wiltshire (1947). The clusterbeans isolate under study is different from A. *brassiciola* in that the conidia and the beaks are much longer. It is also different from *A. cucumerina*, reported on the host by Luttrell (1951), for the same reasons. Details of the morphological characters of the *Alternaria* sp. recorded by Narasimhan (1935) and that of *A. brassicae* listed by Uppal *et al* (1935) on the host are not given by the authors. The fungus under study differs from *A. brassicae* in prossessing much longer beaks than the latter.

Cross inoculation studies have shown that the isolate from clusterbeans can infect several other hosts, besides clusterbeans. *A. solani*, *A. brassicae* and *A. brassicicola* differ from *Alternaria* sp. from clusterbeans in their infectivity to various hosts. Though *A. solani* could infect clusterbeans to some extent, *A. brassicae* and *A. brassicicola* failed to infect the host. *A. solani* differs from the clusterbeans isolate in being more infective to egg plant and tomato and less to radish, mustard, and clusterbeans.

The study of the cultural characters of the four isolates has shown that the clusterbeans isolate differs from *A. solani* in not producing the characteristic zinc orange pigment in organic media and also from *A. brassicae* and *A. brassicicola* in not producing the ivy green coloured mycelium on oat meal and potato dextrose agar media. The clusterbeans isolate produces a characteristic quaker drab to greyish mycelium on complex organic media.

Thus it is evident from these results that the organism under study is distinct from the rest of the species of *Alternaria* in its morphological and cultural characters and in its host specificity. It is, therefore, proposed to name the fungus as *Alternaria cyamopsidis* sp. nov.

Alternaria cyamopsidis Rangaswami & Venkata Rao, spec. nov. Maculae griseolae ad brunneas, circulares vel irregulares, diametientes

2–10 mm., zonatae, nonnumquam coalescentes, in inferiore pagina pallide vel grisee brunneae. Mycelium intercellulare et interacellulare, hyalinum, diametiens 3–6 μ. Conidiophori amphigeni, emergentes 2–8 per stomata, septati, olivacei, geniculati, cicatrice prominenti ornati ad singulas geniculationes, 47–78 × 3–6 μ. Conidia usque quaterna in catenulas rectas disposita, pallide olvacea, obclavata, muriformia, septis transversis 6–11, verticalibus vel obliquis 0–5 ornata, ad septa singula depressa, laevia, 62–146 × 12–19μ, rostrata, rostris hyalinis vel pallide olivaceis, 50–140 × 4–6 μ longis.

Typus lectus in foliis viventibus *Cyamopsis tetragonoloba* Taub. in horto Collegii Agricult, Coimbatore, die 15 mensis octobris anni 1953, a G. Rangaswami, et positus in Herbario Mycologico Gubernii, A.C. & R.I. Coimbatore sub numero 2881; positus etiam in Herb. Crypt. Indiae Orient, I.A.R.I., New Delhi.

Alternaria cyamopsidis sp. nov. Rangaswami & Venkata Rao. Spots greyish to dark brown coloured, circular to irregular, 2 to 10 mm. in diameter, zonate, sometimes several spots coalescing; on the under surface the spots light to greyish brown coloured. Mycelium present both inter- and intra-cellularly, hyaline, 3 to 6 μ in diameter. Conidiophores amphigenous, arising in groups of 2 to 8 through the stomata, septate, greyish olive coloured, geniculate with prominent scar at each geniculation, 47 to 78 × 3 to 6 μ. Conidia in straight chains of upto four, light greyish olive obclavate, muriform with 6 to 11 cross septa and depressions along the septation, smooth surfaced, 62 to 146 × 12 to 19 μ, beaked, beaks hyaline to light olive coloured, 50 to 140 × 4 to 6μ.

On the living leaves of *Cyamopsis tetragonoloba* Taub. Agricultural College Orchard, Coimbatore, 15–10–1953, G. Rangaswami, Type (No. 2881) deposited in the Herbarium of the Government Mycologist, A.C. & R.I., Coimbatore and in Herb. Crypt. Ind. Orient, I.A.R.I., New Delhi.

In the present studies it has been found that *A. brassicae*, *A. brassicicola*, *A. solani*, and *A. cyamopsidis* can infect several host. More than one species of *Alternaria* were isolated by the authors from leaf spots on tomato, radish, and datura collected at Coimbatore during 1956. The natural occurrence of various species of *Alternaria* on these and other hosts and the part played by them in the dissemination of the diseases is to be studied in detail. It is possible that there are more than one strain of *A. cyamopsidis* occurring on clusterbeans as indicated by the isolation of non-sporulating strain from the specimens collected from Tanjore. A survey of the Alternaria blight of the crop in various localities to examine the prevalence of different races, if any, would be very valuable.

Summary

A detailed study of the Alternaria blight of clusterbeans (*Cyamopsis tetragonoloba* Taub.) was made. The organism was brought into pure culture and its pathogenicity established. A comparison of the morphological and cultural characters and host range of the fungus was made with

those of *A. solani*, *A. brassicae*, and *A. brassicicola*. The clusterbeans isolate is distinct in its morphological and cultural characters and in its host range; the beak are $\frac{1}{2}$ to $1\frac{1}{2}$ times the length of the spore; the fungus produces purplish grey mycelium on complex organic media; it can readily pass on to some members of Solanaceae and Brassicae. It is proposed to name the fungus as *Alternaria cyamopsidis* Rangaswami & Venktata Rao, sp. nov.

Acknowledgment

The authors are thankful to Shri T. S. Ramakrishnan, retired Government Mycologist, for his interest in these studies and to Rev. Dr. H. Santapau for the latin diagnosis of the new species. The specimens of cabbage leaves, from which the *Alternaria brassicicola* strain used in these studies was isolated, were collected by Srimathi Sowamini Rajagopalan at Ootacamund, the Nilgris. The authors are also thankful to Dr. K. Ramakrishanan, Government Mycologist, A.C. & R.I., Coimbatore for his helpful suggestions on the systematics of the organism.

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THE PEZIZACEAE OF THE MUSSOORIE HILLS - III

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This paper is intended to record more Pezizaceae from the Mussoorie Hills (5,000–7,500 ft. altitude in the North Western Himalayas) as a part of the study of the Fungal Flora of that region undertaken by the senior author and his students (Thind and Batra, 1956: Thind and Sethi, 1956). All the 7 species described here are new records for India, while new combinations are proposed for two of these species.

The numbers of the species are the serial numbers of Pezizoid Flora.

Type collections have been deposited in the Herbarium of the Punjab University. Duplicate material in formalin-alcohol, is at Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland, U.S.A.

15. *Humaria* gregaria* Rehm., Winter, Flora 55 : 508. 1872. (=*Patella gregaria* (Rehm) Seaver, North Amer. Cup-Fungi, p. 176–177, 1928).

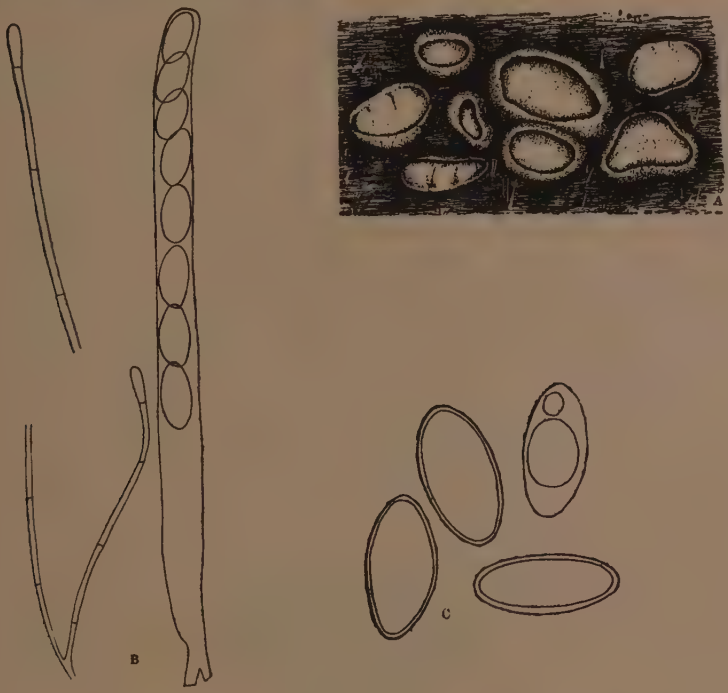
Apothecia up to 3 mm. in diameter, gregarious, often crowded together, sessile, brown to dark brown, closed and globose when young, later opening at the top, expanding and becoming shallow cup-shaped, fleshy-tough: external surface rough, hairy, excipular cells roundish, brown to dark brown: hairs up to 248 x 10.5μ, in clusters, brown, simple, straight, apex obtuse when young, sharp pointed later on when the hairs become bristle-like, multiseptate, thick-walled, wall up to 2.6μ thick; margin incurved, fringed with fascicles of hairs: hymenium concave to almost plane, smooth, whitish with a light bluish tinge.

Asci 210 – 285 x 13 – 18.4μ, cylindrical, apex rounded, tapering below abruptly into a very short stem-like base, do not stain with iodine solution.

Ascospores 21 – 28 x 10.5 – 13μ, 8 in number, some spores degenerated in some asci, hyaline, uniseriate, parallel to oblique, ends overlapping, fusoid, i.e. broader in the middle and usually narrowed at the ends, very inconspicuously verrucose to almost smooth, 1–2 guttulate.

*The generic name *Humaria* is used in place of Seaver's *Patella* for reasons already discussed in the second paper on the series (Thind & Sethi, 1957, *J. Indian bot. Soc.* Vol. 36, p. 20).

Paraphyses up to 300 x 2.5μ, up to 4.2μ at the top, hyaline filiform, usually simple, or sometimes branched, septate, gradually enlarged above.



Text-Fig. 1. *Humaria gregaria* Rehm, A Gregarious apothecia, x 5. B. Ascus and paraphyses, x 400. C. Faintly verrucose to almost smooth ascospores, x 950.

Collected on soil amid mosses under cedrus forest, Kadu Khal, Mussoorie, September 10, 1955, **143**. New record in India.

This collection (n. 143) undoubtedly belongs to *Humaria gregaria* Rehm (=*Patella gregaria* (Rehm) Seaver) and is differentiated by the small but densely gregarious apothecia and inconspicuously verrucose and fusoid ascospores. Its apothecia are much smaller than those of *Humaria hemisphaerica* (Wigg. ex Fr.) Fckl.

16. *Humaria glariosa* (Velenovsky) Comb. nov.

Syn: *Lachnea glariosa* Velenovsky, Monogr. Discom P. 312, pl. 7, f. 24, 1934.

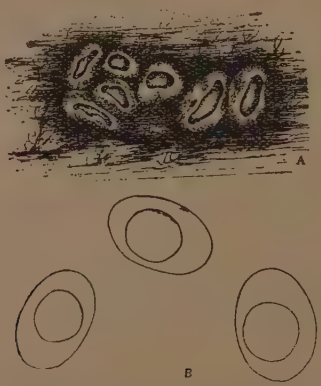
Apothecia upto 2 mm. in diameter, gregarious to congested, external surface brown, hairy, hymenium concave, white with a bluish tinge: asci cylindric, 285 x 21μ: ascospores broadly ellipsoid, hyaline, smooth, uniguttulate, 17.5–23 x 13–17.5μ: paraphyses – 300 x 3.5μ upto 5 μ wide at the top.

Apothecia up to 2 mm. in diameter, gregarious, often crowded and congested together, sessile, regular, often contorted due to mutual pressure, fleshy: external surface light brown due to presence of brown hairs, tubercled excipular cells roundish, brown, up to 26 μ in diameter: hairs usually up to 160 x 14μ light brown to brown, present in fascicles over the tubercles, simple, erect and bristle-like, broader at the base and sharply pointed above, apex acute, septate, thick walled, wall up to 2 μ thick: margin hairy, upturned: hymenium concave, smooth, white with a slightly bluish tinge.

Asci 225 –285 x 15.8 – 21 μ, cylindrical, apex obtuse to truncate, tapering below into a short to long stem-like base, not turning blue with iodine.

Ascospores 17.5 – 23 x 13–17.5 μ, 8 in number uniseriate, parallel to oblique, ends overlapping, hyaline, broadly ellipsoid, smooth, unguttulate, guttule large and filling three-fourth of the spore cavity, guttule smaller in young ascospores.

Paraphyses up to 330 x 3.5 μ, up to 5 μ wide at the top, hyaline, filiform, slightly enlarged at the top, simple, septate.



Text–Fig.2. *Humaria glaviosa* (Velenovsky) Comb. nov., A. Apothecia, x 5. B. Smooth, uniguttulate ascospores, x 950.

Collected on soil, The Park, Mussoorie, September, 16, 1954, **144.** New record for India.

This species is marked by small, light brown, densely gregarious apothecia, whitish hymenium, hairy and tubercled external surface and broadly ellipsoid, smooth spores with a large gutta. The combination nov. proposed here is due to the fact that *Lachnea* is invalid name since it had

previously been used for a genus of flowering plants as pointed out by Seaver (Mycologia 40 : 498. 1948).

17. *Humaria pygmaea* (Clements) Comb. Nov.

(=*Patella pygmaea* (Clements) Seaver, North Amer. Cup-Fungi, p. 179, 1928).

Syn: *Sepultaria pygmaea* Clements, Bot. Surv. Nebr. 4 : 14, 1896. *Lachnea pygmaea* Sacc. and Sydow in Sacc. Syll. Fung. 14 : 755. 1899.

Apothecia up to 1.5 mm. in diameter, gregarious to crowded, external surface brown to dark brown, hairy, hymenium concave, white with a bluish tinge: asci cylindric, 203 x 15.8 μ : ascospores broadly ellipsoid, hyaline, smooth, uniguttulate, 10.5–15 x 7.8–9.6 μ: paraphyses–225 x 2.6μ only up to 3.5 μ wide at the top.

Apothecia up to 1.5 mm. in diameter, gregarious, often crowded together, sessile, brown to dark brown, when young closed and spherical, later on opening at the top, expanding and becoming shallow cup-shaped, tough: external surface rough, hairy: excipular cells spherical, dark brown: hairs up to 390 x 14 μ, brown, simple, straight, rigid and bristle-like apex blunt to acute, multiseptate, septa at short intervals, thick walled, wall up to 2.5 μ thick: margin incurved, fringed with projecting hairs: hymenium concave, smooth, white with a light bluish tinge.

Asci 150 – 203 x 10.5 – 15.8 μ cylindrical, apex rounded, tapering below into a stem-like base, not turning blue with iodine.

Ascospores 10.5 – 15 x 7.8 – 9.6 μ, 8 in number, uniseriate, parallel to oblique, hyaline to subhyaline, broadly ellipsoid, smooth uniguttulate, guttule large and filling three-fourth to almost whole of the spore cavity.

Paraphyses up to 225 x 2.6μ, up to 3.5 μ wide at the top, hyaline, filiform, simple, or branched near the base, septate, slightly enlarged at the top.

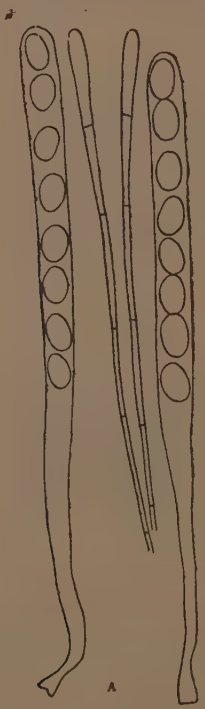
Text-Fig. 3. *Humaria pygmaea* (Clements) Comb. nov., A Apothecia, x 5. B. Ascus, x 400. C. Smooth, uniguttulate ascospores, x 950.

Collected on soil, Kin Craig, Mussoorie, August 24, 1953, **145**. On soil under cedrus forest, Kadu Khal, Mussoorie, September 10, 1955, **146**. New record in India.

This species is common in the Mussoorie Hills and is easily differentiated by the very small, densely gregarious apothecia, whitish hymenium, hairy external surface, and broadly ellipsoid, smooth ascospores with a large gutta. The dimensions of apothecia, hairs, asci, and the ascospores of the Mussoorie collections are somewhat larger for the species. The comb. nov. proposed here is due to the fact that the generic name *Humaria* is used for the first time for the species *pygmaea*. The names *Patella* and *Lachnea* are invalid as already discussed in the second paper on the series (Thind and Sethi, 1957).

18. *Paxina sulcata* (Pers. ex Fr.) Kuntze, Rev. Gen. Pl. **2** : 864. 1891.
Syn: *Peziza sulcata* Pers. Syn. Fung. 643. 1801.
Acetabula sulcata Fuckel, Symb. Myc. 330. 1869.

Apothecia total height up to 3 cm., gregarious, or scattered, stipitate: pileus 1–2 cm. in diameter, shallow to deep cup shaped, regular, fleshy-tough: external surface brown to dark brown, pubescent: pubescent hairs up to 135 x 16μ, hyaline to subhyaline, delicate, short, septate: margin entire: hymenium concave, smooth, deeper coloured than the external surface, i.e. dark brown: stipe up to 2 cm. x 7 mm., lighter coloured than



Text–Fig. 4. *Paxina sulcata* (Pers. ex Fr.) Kuntze, A. Asci and Paraphyses, x 400. B. Smooth, uniguttulate ascospores, x 950.

the external surface, whitish or pale yellow to light brown, of uniform width or slightly narrowed above, deeply longitudinally grooved or sulcated, grooves, running along the entire length or usually only a short distance, thus giving rise to a lacunose appearance, solid, slightly pubescent, pubescent hairs as on the pileus but still less developed.

Asci 262 – 330 x 15 – 18.4 μ, cylindrical, apex rounded, tapering below into a long or short base, not turning blue with iodine solution.

Ascospores 15 – 19.3 x 10.5 – 13 μ , 8 in number, uniseriate, usually parallel, hyaline, broadly ellipsoid, smooth, uniguttulate, guttule large and occupying three-fourth to almost whole of the spore cavity.

Paraphyses up to 338 x 3.5 μ up to 6 μ wide at the top, subhyaline, filiform, clavate, simple, straight, enlarged at the top.

Collected on soil, Jubber Khet, Mussoorie, August 17, 1952 **147**. On soil under cedrus forest, Dhanolti, Mussoorie, September 10, 1955, **148**. New record in India.

This collection undoubtedly belongs to *Paxina sulcata* (Pers. ex. Fr.) Kuntze. The corrugations of its stipe do not extend into the pileus and hence this species is easily distinguished from *Paxina acetabulum* (L. ex Fr.) Kuntze in which case the corrugations of the stipe extend right up to the margin of the pileus.

19. *Paxina hispida* (Schaeff. ex. Fr.) Seaver, North Amer. Cup-Fungi, p. 205–206. 1928.

Syn: *Elvela hispida* Schaeff. Fung. Bavar, **4**. : 108. 1774.
Octospora villosa Hdw. Descr. **2** : 64. 1788.
Peziza stipitata Huds. Fl. Angl. 636. 1798.
Macropodia macropus Fuckel, Symb. Myc. 331. 1869.

Apothecia up to 2 cm. in diameter, usually scattered, sometimes gregarious, stipitate, brown to dark brown, flesh-tough: pileus shallow cupulate when young, later on expanded and folded upward or sometimes downward and appearing saddle shaped: external surface rough, densely hairy or tomentose: haris up to 165 x 14 μ dense, brown, short, delicate, hispid, or tomentum-like, usually in conical fascicles, composed of loosely united moniliform cells which are broader in the middle and narrowed at the ends, straight or bent, simple or branched: margin rough, appearing minutely dentate due to conical fascicles of hairs: hymenium concave to convex, smooth, slightly deeper coloured than the external surface: stipe up to 2 cm. long and up to 5 mm. wide at the base, brown to dark brown, densely hairy like the pileus, broader at the base and gradually narrowed above, up to 1–2 mm. wide at the top, sharply distinguished from the pileus, cylindrical, perfectly even, hairs as on the pileus.

Asci 240 – 300 14 – 17.5 μ, cylindrical, apex rounded, gradually tapering below into a stem-like base, do not stain with iodine solution.

Ascospores 15.8 – 22.3 x 11 – 15 μ, 8 in number, uniseriate, parallel to oblique, hyaline to subhyaline, broadly ellipsoid, or oval to subglobose, smooth, uniguttulate, guttule large and filling whole of the spore cavity.

Paraphyses up to 300 x 3.5 μ, up to 6 μ wide at the apex, filiform, yellowish brown, simple, or branched near the base, septate, enlarged at the top.

Text-Fig. 5. *Paxina hispida* (Schaeff. ex. Fr.) Seaver, A. Ascus and paraphyses, x 400.

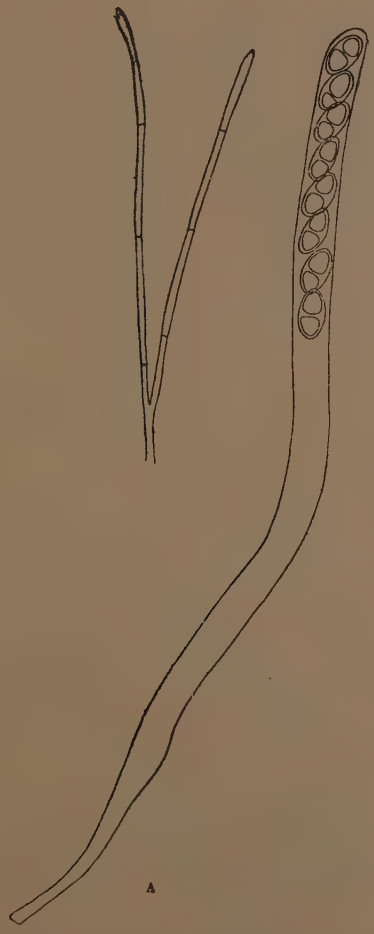
Collected on soil under cedrus forest, Dhanolti, Mussoorie, August 27, 1955, **149**. New record in India.

This species is easily distinguished from *Paxina acetabulum* (L. ex. Fr.) Kuntze. and *P. sulcata* (Pers. ex. Fr.) Kuntze in its even stipe. The spores of the Mussoorie collection are rather small for the species and contain only one large guttule filling whole of the spore cavity.

20. *Geopyxis catinus* (Holmsk. ex. Fr.) Sacc. Syll. Fung. **8** : 71. 1899.

Syn: *Peziza catinus* Holmsk. Otia **2** : 22; 1799.
Pustularia catinus Fuckel, Symb. Myc. 328. 1869.
Aleuria catinus Gill. Champ. Fr. Discom. 39. 1879.

Apothecia up to 2 cm. in diameter and up to 1.5 cm. deep, gregarious, or crowded, shortly stipitate to subsessile, deeply cupulate, at first globose, regular, fleshy, brittle: external surface pale yellow, densely but finely pubescent, strongly pubescent at the margin: pubescent hairs up to 150 x 11 μ. hyaline, short, septate, narrow: margin crenate and infolded, densely pubescent: hymenium deeply concave, smooth, concolorous with the external surface: stipe up to 7 x 3.5 mm., concolorus with the pileus, lacunose to nearly even, solid, densely pubescent, pubescent hairs longer and more conspicuous than those on the pileus, hyaline, septate.



Text–Fig. 6. *Geopyxis catinus* (Holmsk. ex. Fr.) Sacc. A. Ascus with a very long stem like base and a branched paraphysis, x 400.

Asci 300 – 375 x 12 – 16 μ, elongate-cylindrical, apex rounded, tapering below into a very long stem-like base, not turning blue with iodine solution.

Ascospores 17.5 – 23 x 10.5 – 12.3 μ, 8 in number, uniseriate,usually oblique, ends overlapping, hyaline, broadly ellipsoid, rarely with ends slightly narrowed, smooth, biguttulate, guttules large.

Paraphyses up to 400 x 3 μ, up to 4 μ wide at the top, hyaline, filiform, usually branched near the base, slightly enlarged at the top, septate.

Collected on soil under Oak forest, Dhobi Ghat, Mussoorie, September 4, 1953, **150**. On soil under Oak forest, Jubber Khet, Mussoorie, September 10, 1954, **151**. New record in India.

These collections (no. 150 and no. 151) undoubtedly belong to *Geopyxis catinus* (Homsk. ex. Fr.) Sacc. and are marked by pale yellow, deeply cupulate and shortly stipitate apothecia, pubescent external surface, crenate margin, asci with long stem-like base, and smooth, ellipsoid ascospores with two large oil drops. The apothecia of the Mussoorie collections are not varrucose on the outside as reported for the species.

21. *Peziza pustulata* Pers. ex. Fr. Syn. Fung. 646. 1801.

Syn: *Octospora pustulata* Hedw. Descr. 2 : 19. 1787.
Scodellina pustulata S.F. Gray. Nat. Arrang. Brit. pl. 1 : 668. 1821.
Plicaria pustulata Fuckel, Symb. Myc. 327. 1869.
Aleuria pustulata Gill. Champ. Fr. Discon. 45.1879.
Galactinia pustulata Boud. Hist. Class. Discom. Eu. 48. 1907.

Apothecia 1 – 4 cm. in diameter, up to 1.5 cm. deep, gregarious, often crowded together and caespitose, sometimes scattered, sessile, narrowed below into a stem-like base, deep cup shaped, globose when young, later expanding and becoming deep to shallow cupulate, regular, or much contorted, fleshy, brittle, not turning golden yellow when broke: external, surface dirty white with a pale yellowish tinge, strongly pustulate (fluffy small cottony balls spread all over the surfaces as seen under the low power of microscope) pustules more conspicuous in young specimens, becoming less prominent in mature ones: margin entire, sometimes, wavy, looking crenate due to the presence of pustules on the outer face but is perfectly smooth on the inner hymenial face: hymenium concave, smooth, light to deep brown.

Asci 240 – 327 x 12 – 14 μ cylindrical, apex typically truncated tapering below into a stem like base, turning blue with the iodine solution.

Ascospores 14.8 – 18.4 x 9.6 – 12.3 μ, 8 in number, uniseriate, parallel to oblique, ends usually overlapping, subhyaline, ellipsoid, conspicuously verrucose, warts abundant and up to 0.8 μ long incomplete or

separate reticulations also observed in surface view probably due to the union of some warts, uniguttulate, guttule large and occupying three-fourth of the spore cavity.

Paraphyses up to 338 x 5 μ, up to 10 μ wide at the apex, hyaline to subhyaline, filiform, simple, septate, considerably enlarged at the top.

Text–Fig. 7. *Peziza pustulata* Pers. ex. Fr., A. Ascus with a truncate apex and top of paraphysis, x 400.

Collected on soil under cedrus forest, Dhanolti, Mussoorie, August 26, 1955, **152**. New record in India.

This species is characterized by strongly pustulate, whitish apothecia. The Mussoorie collection (no. 152) was collected on soil and not on burnt ground as reported for the species.

ACKNOWLEDGMENTS

The authors are deeply indebted to Miss Edith K. Cash of U.S. Dept. Agr., Beltsville, Maryland, for help in the identification of the species and valuable suggestions and Prof. P. N. Mehra for encouragement and facilities.

PLATES

Fig. 1

Fig. 2

Fig. 3

Fig. 4

They are also thankful to Mr. B. Khanna for making illustrations of the fructifications,

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Punjab University, Amritsar.

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Explanation of Plate

Fig. 1. *Paxina sulcata* (Pers. ex. Fr.) Kuntze, A pileate fruit body with prominently sulcate stipe.

Fig. 2. *Paxina hispida* (Schaeff. ex. Fr.) Seaver, A Pileate fruit body with even stipe.

Fig. 3. *Geopyxis catinus* (Holmsk. ex. Fr.) Sacc., Short stipitate to subsessile, deeply cupulate apothecia with a crenate margin.

Fig. 4. *Pezia pustulata* Pers. ex Fr., A large fully expanded apothecium.

PHYTOPHTHORA BLIGHT OF SESAMUM

G. B. Kale and N. Prasad.

(Accepted for publication June 30, 1957)

Sesamum (*S. orientale* L.) is an important oilseed crop of India, Burma, Egypt and some of the Mediterranean countries. Gujerat is an important region for production of this oil-seed. Its oil is highly prized as a cooking medium in this region. This crop occupies roughly an area of a hundred thousand acres every year. It is generally sown in the beginning of July and harvested by the end of September or early October. Two varieties of this crop are grown in this area viz. *tal* and *tali*. The former is grown as mixed crop with *kodra* (*Paspalum scrobiculatum* L.), cotton (*Gossypium arboreum* L. var *typicum* H. & G.) castor (*Ricinus communis* L.) and pigeon pea (*Cajanus cajan* L.) while the latter is grown as a sole crop.

During recent years, sesamum crop has been observed to suffer annually from a serious blight disease at the Institute of Agriculture. Anand and an adjacent farmers' fields. The disease is characterized by appearance of water-soaked spots on the leaves, which extend in size and result in destruction of leaves. Similar water-soaked spots can also be observed on the shoot. (Fig. 2). The lesions so formed are brown in the beginning but later turn black. At this stage, affected plants can easily be distinguished from healthy ones even from a distance (Fig. 1). The discolouration is very marked near the base of the stem adjacent to soil. Pods on the affected branches are poorly formed. The seeds are shrivelled and in case of severe attack they turn brown. With continuance of humid weather a wooly growth of the fungus can be seen on the pods bearing caenocytic mycelium with papillate zoosporangia.

Butler (1918) recorded *Phytophthora parasitica* Dast. on Sesamum in India. Cifferi (1930) reported occurrence of *P. parasitica* from Puerto Rico. Patel, Kamat and Bhide (1949) again reported it from India. Patel, in a personal communication, mentioned that this record concerned with pathogenicity trials with an isolate of *P. parasitica* from *Psidium guyava* L. Crandall and Dieguez (1948) have recorded *P. cactorum* causing stem canker on sesamum from Peru while Frezzi (1950) reported *P. parasitica* from Argentina.

From a review of papers published on the subject, there does not seem to be any record of such a serious disease of sesamum from any country. It was therefore thought desirable to carry out a preliminary survey of the crop on the Institute farm and fields of neighbouring villages. The plots were selected at random and healthy and diseased plants were counted in a patch and later percentage of infection worked out. Observations were taken for two years and the percentage of infection during these two years has been recorded in Table I. Observations were recorded on the two locally cultivated varieties, viz. *tal* and *tali*.

Fig. 1. Symptoms produced by the disease at the base of the stem.

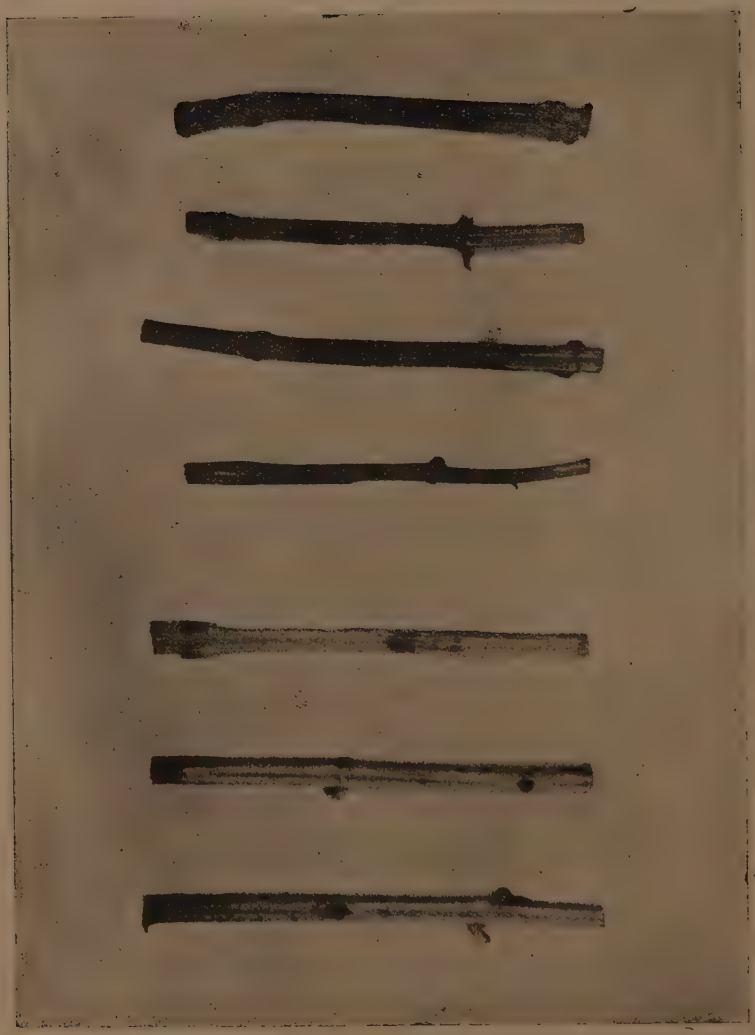

Fig. 2. Symptoms of the disease on stem.

In 1952 and 1953, crop was examined in the Farm area and villages and the disease intensity was found to vary between 1 to 66 per cent. Significantly less percentage of infection was found in case of *tal*. The reason for the difference in the incidence of two varieties most probably lies in the method of cultivation of the two. Since *tal* is always grown as a mixture with other crops, the plants are at a considerable distance from each other and less liable to be infested by contact with a diseased plant.

To get an idea of the reduction in yield of seed by the disease, a number of affected patches were marked and the observations on the toal area and the yield obtained were recorded. The data so obtained have been presented in Table 1.

TABLE I. REDUCTION IN YIELD DUE TO DISEASE.

S. No.	Area of plot in Acres	Infection percentage	Expected yield	Yield obtained in lbs.	Percentage of loss
1.	2.00	66	800	288	64
2.	0.65	65	260	90	63
3.	0.67	55	270	131	52
4.	0.65	24	270	216	20
5.	1.50	12	600	520	13

The average yield of *tal* in the area is found to be about 400 lbs. per acre.

Since the disease generally kills all the affected plants, it can be observed that net loss in yield is directly proportional to the incidence of the disease.

ISOLATION: Isolations were made from diseased leaves, petioles, inflorescence and stems. In almost all cases, a white fluffy mycelium appeared on agar slants. The mycelium under microscopical examination was found te be aseptate which formed zoosporangia readily when floated in sterilized tap water.

PATHOGENICITY TRIALS: Sesamum seedlings were grown in earthern pots of 9 x 8 inches size in sterilized soil. Inoculation was carried out by removing a little soil from the base of the plants, and little portion of a vigorous culture on oat meal medium was placed near the stem. This was then covered with soil. In case of control pots, a piece of oat meal medium was placed near the stems. Pots were then watered twice or thrice daily.

Symptoms appeared on seedlings in nine days after inoculation. The first visible symptom was the appearance of soft water soaked brownish discolouration at the base of the stem. The discolouration gradually extended upwards and a black streak could be observed on the sixth day after the appearance of first visible symptom. The affected plants were killed in the next three to four days. Isolations were made from the affected

seedlings? readily formed sporangia when floated in sterilised tap water. Under artificial infection both varieties of locally grown sesamum were equally susceptible to the disease.

Leaves and inflorescence were also inoculated by placing a small piece of medium with mycelium of the fungus. Water soaked patches could be observed on the leaves four days after inoculation. These spots gradually extended and leaves withered away in a week after the appearance of the first symptoms. Symptoms similar to those appearing on stems were found on inflorescence as well.

HOST RANGE: In order to determine whether the fungus isolated from diseased sesamum plants, is capable of infecting plants other than sesamum grown during the same season, a number of crops were selected. They were *Crotalaria juncea* L., *Cyamopsis tetragonoloba* Taub., *Nicotiana tabacum* L., *Lycopersicum esculentum* L., *Solanum melongena* L., *Capsicum annum* L., and *Ricinus communis* L. *Sesamum orientale* (both *tal* and *tali* varieties) was inoculated to serve as a control.

Four plants of each of the above mentioned crops were grown in pots filled with sterilized soil. Each of them were inoculated with the culture of the fungus in a manner described as before. The fungus failed to infect any crop other than sesamum. No symptoms could be observed in other crops even after two months of inoculation. The experiment was repeated with similar results thereby showing conclusively that the fungus is restricted in its parasitism to sesamum only.

MORPHOLOGY: The mycelium in young cultures is caenocytic and profusely branched but septa can be observed in two months old cultures. The hyphae are hyaline and are 2–8 μ in thickness. Optimum temperature for growth is about 28° C.

The fungus does not form sporangia on culture medium but abundant sporangia can be observed in nature on wooly mycelium growing on infected pods under humid conditions. Sporangia can also be observed in one month old cultures. On the host the mycelium emerges out after rupturing the epidermis and the sporangiophores are gnerally of the same thickness as the mycelium. The sporangiophores are branched sympodially and bear ovate to spherical sporangia terminally (vide Figure 3). They have a prominent apical papilla. Hundred sporangia were measured at random and they have been grouped according to their size in Table No. 11.

TABLE II. SIZE OF ZOOSPPORANGIA.

S. No.	Range of group in microns.		No. of sporangia in a group.
	Length	Breadth	
1.	25—30	20—25	10
2.	30—35	25—30	21
3.	35—40	30—35	45
4.	40—45	30—35	26
5.	45—50	30—35	8

Fig. 3. Sporangia of *Phytophthora parasitica* var *sesami*.

Fig. 4. *Oospores* of *P. prasitica* var *Sesami* (In 2 months old culture).

The average size of sporangia was found to be 38.5 x 33.0 μ.

The mycelium when floated in tap water formed zoosporangia readily in about 48 hours. The sporangia so formed agreed in shape and size to those obtained on the plants during very moist weather. After formation of the sporangia, zoospores were formed inside and they clearly separated from each other within the sporangium. These zoospores were liberated in water if the mycelium was flooded with fresh supply of water. The fungus forms oospores abundantly in cultures more than two months old. The oospores are spherical, smooth, double-walled and are hyaline in colour. Oospores can be observed in infected portions of the stem as well. The antheridium can be observed at the base and the attachment is typically amphigynous (vide Figure 4).

The fungus was grown on different media viz. oat meal agar, corn-meal agar, potato dextrose agar, Sabouraud's agar, Richards' medium with ammonium sulphate and with potassium nitrate. Best growth was obtained on oatmeal agar. These media were kept at three temperatures viz. 25°C, 30°C, and 35°C. The diameter of the colonies in Petri dishes was measured after 96 hours and the results are recorded in Table III.

TABLE III. GROWTH OF ISOLATE FROM SESAMUM ON DIFFERENT MEDIA AT THREE TEMPERATURES.

S. No.	Name of the medium	Average growth in mm. after 96 hours.		
		25°C	30°C	35°C
1.	Oat meal agar.	70	80	75
2.	Corn meal agar.	63	68	65
3.	Potato dextrose agar.	43	51	44
4.	Sabourad's agar.	16	18	16
5.	Richards' medium with ammonium sulphate.	14	20	18
6.	Richards' agar with potassium nitrate.	12	17	12

It can be readily seen from the above table that oat meal agar produced best growth when compared with other media. Best growth was found at 30°C in all media. Even at 35°C good amount of growth was produced in all cases thereby showing that this fungus can thrive well at that temperature.

TAXONOMY: Various workers have tried to evolve a sound basis for the identification of species of *Phytophthora*. Rosenbaum (1917) identified a small number of species by the types of antheridia and on the average size of sexual and asexual reproductive organs but recent study has shown that not much reliance can be put on the size of the reproductive organs as wide variations within a species are known to occur.

Leonian (1934) presented a key for identifying a number of species on the basis of their physiological reactions, particularly, formation of repro-

ductive organs on various nutrient media. He studied the influence of the host on the morphology of sporangia and concluded that the identification of species cannot be reliably made by cultural characteristics from the host and they should be made from cultures grown under controlled conditions. He found considerable variation among strains derived from the same parent culture and inferred that size of sporangia, oogonia, oospores and antheridia could not be relied upon as criteria for differentiating species of *Phytophthora*.

The only character that can be relied upon for classifying *Phytopththora* seems to be temperature growth relationship and Tucker (1941) in his monograph has principally used this character for identifying various species.

Tucker recognises two species which can grow at a temperature of 35°C viz. *P. parasitica* Dast. and *P. capsici* Leon. (1941). The latter is pathogenic on chillies, brinjal and tomato. Since our isolate failed to infect any one of these crops, it is quite reasonable to assume that it cannot be *P. capsici*.

Taking into consideration the morphological characters viz. sessile sporangia with a prominent papilla and possession of amphigynous antheridia, it can definitely be concluded that it belongs to *P. parasitica*. This identification is strengthened by temperature growth studies. As the isolate, in repeated trials, failed to infect any host other than sesamum, it is concluded that it is highly specialized in its parasitism and on those grounds it should be known by a trinomial viz. *P. parasitica* var. *sesami* Prasad (n. var).

The culture of this fungus has been deposited in the culture collection in the Mycological Section, Indian Agricultural Research Institute, New Delhi and Commonwealth Mycological Institute, Kew, England.

Discussion

Although *P. parasitica* Dast. has been recorded in the past by several workers from different countries, no one found it to be present in virulent form. Butler recorded it only as a leaf blight. Under the present studies, it has been found to destroy a substantial part of the crop.

Thomas and Ramkrishnan (1948) have merged *P. parasitica* with *P. colocasiae* but it seems their suggestion has not been accepted as such. *P. parasitica* appears in modern literature as a valid species and we have accepted the present position that it can be recognised as such. The isolates of this species are specialized on tobacco and piper betel and new varieties are recognized on the basis of their specialization on a particular host. Keeping this in view, we propose to call it as *P. parasitica* var *sesami*.

Summary

A very destructive disease was found on *Seasamum orientale* in Gujerat. The incidence of the disease was found to be as high as 66 per cent in the two cultivated varieties *tal* and *tali*, both of which were found to be equally

susceptible. The fungus isolated from diseased plants failed to infect any host other than *Sesamum*. On the basis of the study of morphological characters and growth temperature relationship, the pathogen was identified as *P. parasitica* Dast. Since it is highly specialized in its parasitism, a new name *P. parasitica* var *Sesami* is assigned to it.

ACKNOWLEDGMENTS

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MARKET DISEASES OF FRUITS AND VEGETABLES IN KUMAON

I. COTTONY LEAK OF BEANS

K. S. Bhargava and S. C. Gupta

(Accepted for publication June 30, 1957)

During the summer of 1955 dwarf bean pods were seen with water-soaked lesions and white cottony mycelium in Nainital, a hill sation, 6400 ft. above sea level. In advanced cases some of them showed leaky condition. Cottony leak may be caused by several species of *Pythium*. Harter and Whitney (1927) reported that *Pythium aphanidermatum* (Eds.) Fitz. causes nesting of snap beans in transit similar to that caused by *P. ultimum* Triow, *P. splendens* and *P. myriotylum* Drechsl. Ramsey and Wiant (1941) reported *P. butleri* Subr. as causing cottony leak disease of beans in U.S.A. *Pythium debaryanum* Hesse causing decay of beans in transit has not so far been reported in India.

The disease as it occurs on dwarf variety of beans is characterised by the production of large water-soaked lesions which enlarge greatly. The causal organism grows profusely over the surface with abundant cottony mycelium and soon a dirty fluid is seen oozing out of the infected pods and is known as "cottony leak". Microscopic examination revealed the presence of non-septate hyphae and numerous oogonia and antheridia in a all stages of development.

Isolations were made on oat meal agar from surface sterilised infected pods out of discoloured internal tissue below the water-soaked lesions and also from portions of aerial mycelium. Formation of sporangia was induced by transferring bits of mycelium grown in 1% peptone solution to sterilised distilled water. Sex organs were readily produced by growing the fungus on hemp seeds in water.

Morphology of the fungus: Hyphae branched, usually 4.5 μ – 5.5 μ in diameter. Sporangia spherical to oval, terminal or intercalary 15.5 μ to 25 μ in diameter, germinating by germ tubes or by zoospores. Oogonia smooth, terminal or intercalary, spherical 16 μ to 24 μ average 20.7 μ in diameter. Oospore smooth, aplerotic 15.5 μ to 20 μ average 17.25 μ in diameter. Antheridia one or more per oogonium, monoclinous or diclinous.

These characters are in close agreement with those of *Pythium debaryanum* Hesse with which several species are considered synonymous (Middleton, 1943).

Inoculation experiments: Six sound bean pods were surface-sterilised and a small incision was made with a sterilised scalpel in each. Small pieces of mycelium from a pure culture of the fungus were deposited on the incised portion. Side by side some mycelium was also deposited on sound pods without any incision. Treated beans were then placed under a

bell jar at room temperature (19°–21°C). Infection took place in all the pods in which incisions had been made, and the fungus was reisolated.

In another experiment some surface-sterilised sound pods were placed side by side touching the infected pods. These did not show any infection even after a week showing thereby that the fungus is a wound parasite and is unable to attack sound tissues.

In a third series of experiments inoculated pods were incubated at four different temperatures. The infection spread very quickly at 20°C and 25°C but slowly at 12°C and 30°C. The optimum temperature for the spread of the disease was found to be 20°–25°C.

In cross-inoculation studies it was found that fruits of *Luffa aegyptica Coccinia indica, Citrullus vulgaris fistulosus* and *Hibiscus esculentus* completely rot in 4–5 days, whereas those of *Capsicum annuum, Solanum melongena* and *Momordica charantia* take 7–9 days.

CONTROL MEASURES: Species of *Pythium* are prevalent in most soils. Pods of french beans dwarf variety rest on the ground or are very near the ground during the growing period. It appears that initial infection takes place on pods resting on the ground or the spores of the fungus reach the pods by splashings from the soil. The spores of the fungus are undoubtedly carried from the soil on the pods before they are picked up. From these primary infections the fungus spreads to other pods kept in baskets.

To avoid infection from spreading, all pods showing water soaked lesions should be discarded at the time of packing. Moreover the pods collected from fields known to be infested should be surface dried as soon as possible. Since the fungus is unable to attack sound pods, segregation of any cracked or broken pods from the rest will largely eliminate the disease. The suspected beans should be kept away from other vegetables.

SUMMARY

During the summer of 1955 a serious disease of stored dwarf beans was noticed. The causal organism was identified as *Pythium debaryanum*. It is a wound parasite. The infection spreads very quickly at 20° to 25°C. Careful handling and other sanitary measures would help in eliminating the disease.

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FIELD TESTING OF FUNGICIDES

P. Govindarao and J. Subbaiah.

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During the year 1954–55 fungicides namely Fungimar, Cupravit, Wettable sulphur, Wettable ceresan, Mercury Spray Bayer, Dithane Z. 78 and T.B. 4452 b were received from different firms for a test of their efficacy. Attempts were made to evaluate these fungicides in controlling the post-emergence damping off disease occurring in the Bapatla soils.

Fungicides are generally tested in the laboratory by using the glass slide, test tube dilution, or Petri dish methods. But sometimes such methods do not give the same results as when the fungicides are subjected to field tests (Zentymer 1955). The object of the present studies was to test some of the proprietary fungicides for their efficacy, and present a rapid field testing method.

Experimental: Damping off disease incited by *Pythium aphanidermatum* (Eds.) Fitz. which commonly occurs in the nurseries of tobacco and chillies was chosen for testing these fungicides under identical sets of conditions The ryots do not know the pre-emergence damping off, but are quite anxious to control post-emergence damping off which is normally found to occur between the 9th and 12th day after sowing in chillies. So, the spraying was started either on the 12th day or earlier depending on the incidence of the disease. But in the case of the tobacco nurseries the seedlings are under the cover of the casuarina trash upto the middle of the third week. Observations on the post emergence damping off are made only after the casuarina cover is removed, and the spraying was resorted to from that date. The fungicides are tested for the control of a part of the post emergence damping off only. Field experiments were conducted in randomised, replicated lay outs. On each of the crops duplicate experiments were conducted with an interval of one month to allow for the seasonal influence on the intensity of the disease. The details of the fungicides and their doses at which they were sprayed are given below. These were compared with Bordeaux mixture. The fungicidal treatments were given thrice at 10 day intervals starting from the 21st day after sowing for the tobacco nurseries and twice from the 12th day for the chilli nurseries. To assure infection, the seedlings were sprayed with pure culture of *Pythium aphanidermatum* (Eds.) Fitz. two days after each fungicidal spray.*

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*Suitable controls were maintained without spray treatments.

No.	Name of the fungicide.	Dose.	Active ingredient
1.	Bordeaux mixture	1%	
2.	Fungimar.	@0.4% or ¾ oz. per galllon	Cuprous oxide containing 50% metallic copper (Bharat Pulverising Company Bombay).
3.	Cupravit.	do	Contains 50% copper as Copperoxychloride (Chikka Ltd. Bombay)
4.	Wettable ceresan	@0.31% or ½oz. per gallon	Contains 2.5% Methoxy-ethyl-Hg - chloride as main ingredient (Chikka Ltd., Bombay)
5.	Mercury spray Bayer.	@0.2% or ⅓ oz. in 1½ gallons.	Contains on organic mercury salt (Chikka Ltd., Bombay)
6.	T.B. 4452 b.	@ 0.31% or ½oz. per gallon.	Contains as active ingredient a new mercury free organic compound (Chikka Ltd. Bombay)
7.	Dithane Z 78.	-do-	Contains 65% zinc ethylene bisdithiocarbamate. (Amritlal & Co., Bombay).
8.	Wettable Sulphur	@ 0.62% or 1 oz. per gallon.	Contains 95% pure sulphur (Bharat Pulverising Co.)

The first series of these trials was started in July 1954. Beds were prepared and farmyard manure was applied as a basal dressing and the seeds of tobacco & chillies were sown on 12-7-54 and 13-7-54, respectively. The tobacco beds were given a covering of casuarina trash, as is the usual custom in the locality for conserving moisture and for avoiding disturbance of seeds during watering The chilli beds were given a covering with paddy straw. Watering was done with rose-can for about 3 weeks in the tobacco nurseries and for two weeks in the chilli nurseries. Later, splash watering was resorted to for both the nurseries and the covering on the seed beds was also gradually removed. The first fungicidal treatment was given generally on the 21st and 12th days after sowing for the tobacco and chillies nurseries, respectively. Two days after, a spray of the pure culture of the fungus *Pythium aphanidermatum* was given to both the nurseries. During the period under study the chilli nurseries received two fungicidal sprays at intervals of 10 days and two sprays with fungal culture, while the tobacco nurseries received three fungicidal sprays and three sprays with fungal culture.

Damping off appeared at the beginning of the 4th week in the tobacco nurseries and at the end of the 2nd week in the chilli nurseries. Daily counts of dead seedlings were recorded starting from the day of incidence of the disease till about 10 days. The dead seedlings were removed from the beds either by using forceps or with the hand, and examined for the presence of *Pythium aphanidermatum*.

Seedlings that could stand transplanting were pulled out in one lot between the 45th and 50th day after sowing, in the case of chillies, while for tobacco the seedlings were pulled out in three stages i.e. on the 45th, 60th, and 80th day after sowing. The total number of seedlings in either case were recorded for each bed separately and the percentages of damped-off seedlings were calculated. It is on this basis that the results of fungicidal effect are evaluated.

A duplicate of the above experiments was started and the beds were sown on 7-8-54 and 10-8-54 for chillies and tobacco respectively. The same procedure as detailed earlier was followed in this case also and the data recorded. The data for all the four experiments were analysed statistically and the results are given in table I. (page 53).

Results and Discussions

From a perusal of the data, it is observed that all the fungicides tried proved significantly superior to control in two out of the four experiments. Bordeaux mixture 1%, Cupravit and Fungimar proved superior to control in all the four experiments and were on a par among themselves in all the experiments. Mercury spray Bayer, was on a par with the control in two out of the four experiments while Wettable Sulphur, T.B. 4452 b, Dithane Z, 78, and Wettable Ceresan were on a par with the control in one experiment. While Cupravit and Fungimar were on a par with the standard Bordeaux mixture 1% in all the experiments, T.B. 4452 b, Dithane Z 78 and Wettable Ceresan were on a par with it in two experiments, and Wettable Sulphur and Mercury Spray Bayer were on a par with it in only one experiment. Dithane Z78, Wettable sulphur, T.B. 4452 b, Wettable Ceresan and Mercury Spray Bayer were on a par in two experiments while they showed differences in the other two. It may be inferred in a general way that the two copper fungicides, Cupravit and Fungimar proved superior to the other proprietory fungicides tried.

In order to evaluate all the fungicides as against the control and between themselves the averages of the mean percentages of incidence of damping off in all the four experiments have been struck and their order of merits has been presented below.

Order of merit of the different fungicides.

S.No.	Fungicide tried.	Mean % incidence of damping off (Mean of all the four experiments.)	Order of merit
1.	1% Bordeaux mixture	22.17%	1
2.	Fungimar	27.21%	2
3.	Cupravit	27.48%	3
4.	Wettable sulphur	34.75%	4
5.	Dithane Z, 78	35.51%	5
6.	T.B. 4452 b	37.13%	6
7.	Wettable ceresan	38.38%	7
8.	Mercury spray Bayer	40.43%	8
9.	Control	53.96%	9

TABLE I

Experiment 1. (Chilli - July sowing)

Conclusions:—

	B. Mixture 1%.	T.B. 4452 b.	Wettable Sulphur.	Wettable Ceresan.	M.S. Bayer.	Cupravit.	Dithane Z. 78.	Fungimar.	Control
Mean % of damped off seedlings.	8.48	9.90	11.18	11.65	12.08	13.14	13.26	14.42	24.78

S.E: 2.52; C.D.: 7.20; G.M.:13.21. (P=0.05)

Experiment 2. (Chilli - August sowing)

	Cupravit.	B. Mixture.	Fungimar.	Dithane Z. 78.	Wettable ceresan.	T.B. 4452 b.	Wettable sulphur.	M.S. Bayer.	Control
Mean % of damped off seedlings.	14.03	15.53	16.42	16.43	21.42	21.54	23.59	23.74	29.75

S.E: 2.516; C.D.: 7.19; G.M.: 20.27 (P=0.05)

Experiment 3. (Tobacco July - sowing)

Conclusions:—

	B. Mixture.	Cupravit.	Fungimar.	Wettable sulphur.	T.B. 4452 b.	Dithane Z. 78.	Wettable ceresan.	M.S. Bayer.	Control.
Mean % of damped off seedlings.	38.13	48.24	48.32	62.44	66.44	67.15	67.39	69.01	79.48

S.E: 5.02; C.D: 14.36; G.M: 60.74 (P=0.05)

Experiment 4. (Tobacco August - sowing)

Conclusions:—

	B. Mixture 1%.	Fungimar.	Cupravit.	Wettable sulphur.	Dithane Z. 78.	T.B. 4452 b.	Wettable ceresan.	M.S. Bayer.	Control.
Mean % of damped off seedlings.	26.56	29.69	34.51	41.81	45.19	50.67	53.06	56.90	81.84

S.E: 3.44; C.D: 9.83; G.M.: 46.69. (P=0.05)

From a perusal of the above table it is observed that the Copper fungicides are better than the others tried. Wettable Sulphur comes next to the copper fungicides. Compared to the Copper fungicides wettable sulphur is cheap. While the former fungicides sell at about the rate of Rs. 2-8-0 per lb. the wettable sulphur sells at Rs. 1– per lb. Hence it may be desirable to test wettable sulphur at higher doses to see if it would favourably compare with the copper fungicides.

The test fungus *Phthium aphanidermatum* selected for the conduct of these experiments is a very common soil-borne organism available almost in all the soils in this State. The fungus can also be easily cultivated in the laboratory and can be used for creating uniform infection in case natural infection is lacking. The crops selected are nurseries of chillies and tobacco which could be raised quickly within about 50 and 80 days respectively. Hence it is quite easy to conduct a trial in randomised replicated plots within about 80 days. And a second series could be started a month after the starting of the first one so that one could easily get the results of four experiments within about four months. The area required for the experimental trials is not much. To test ten new fungicides about four cents of area would be enough for each trial. New fungicides have to be field tested before they could be recommended to the cultivators.

Further work on other useful test fungi and susceptible plants is in progress.

Summary

Seven fungicides were tested for their efficacy in controlling the post-emergence damping off disease caused by *Pythium aphanidermatum*. Four experiments were conducted, two on chillies and the other two on tobacco. All the fungicides except M. S. Bayer proved superior to control in three out of the four experiments. None of the fungicides tried was found phytotoxic to the seedlings of chilli and tobacco on which the experiments were conducted.

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STUDIES ON THE DEVELOPMENT AND CONTROL OF FRUIT ROT OF CHILLIES

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Introduction

Ripe fruit-rot of chillies (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) Butler and Bisby has been found to be quite serious and widespread in Assam. It has been observed throughout the State wherever chillies are grown. From 12 to 32 per cent of the fruits are affected by this disease.

As the disease causes severe damage in Assam, certain studies were carried out during the years 1950 to 1953 to determine the conditions which help its development, and to devise suitable methods of control. Results of these studies are reported in this paper.

Ripe fruit-rot of chillies is also known to occur in other parts of the world. It has been reported from Bihar by Dastur (1921), from Porto Rico by Seaver, Chardon and Toro (1932), from Ceylon by Bertus (1927), from Tanganyika by Wallace (1929) and from Georgia byHiggins (1934).

Symptoms

Only the ripe fruits which have turned red become affected. The first outward sign of the infection is the appearance of a small black circular spot, generally sharply defined but at times diffused. The disease does not spread concentrically. It spreads more in the direction of the long axis of the fruit, so that the orginally circular spot becomes more or less elliptical. As the infection progresses, the spot gets either diffused and black, greenish black or dirty grey in colour, or is markedly delimited by a thick and sharp black outline enclosing a lighter black or straw-coloured area. Two or more diseased spots may coalesce, thereby masking the regularity of the individual spots, but the delimiting black line is not always completely obliterated where the infected areas have united. Badly diseased fruits lose their normal red colour and turn straw-coloured or, in some cases, pale white. The acervuli are generally densely gregarious or scattered all over the infected parts; at times they are concentric. They project a little above the surface of the fruit, and are bristly and carbonaceous. The spores ooze out of the acervuli in pink masses or cirrhi under moist conditions. Plate I shows the symptoms of the disease.

When a diseased fruit is cut open the lower surface of the skin is found covered with minute, black, spherical elevations; these are the stromatic masses or sclerotia of the fungus. In advanced cases, the seeds are covered

Ripe fruit rot of chillies; Symptoms of the disease.

by a felt of white mycelium, in which are embedded a few black or grey-green stromatic bodies. Infected seeds turn rusty in colour. No green fruit is affected by the disease.

Growth of the Fungus in culture in relation to Temperature, Humidity and Hydrogen-on Concentration.

These studies were carried out with the pure culture of *C. capsici* which was obtained from single spore isolation.

(i) *Temperature.* The growth of the fungus in relation to temperature was studied by growing it on potato dextrose agar and incubating the inoculated Petri dishes at different temperatures. Five Petri dishes were kept at each temperature and the rate of growth measured every day. The average diameter of the colonies observed after 5 days is presented in Fig. 1. From the data presented in Fig. 1 it would appear that the optimum temperature for the growth of the fungus lies somewhere around 28 °C.

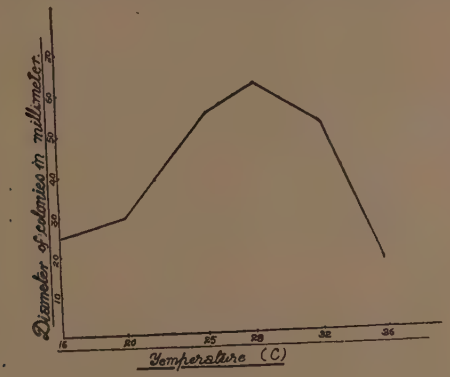

Fig. 1. Growth of *Colletotrichum capsici* in culture in relation to temperature.

(ii) *Humidity.* The effect of humidity on the growth of the fungus was determined by exposing cultures of the fungus to atmospheres with different degrees of humidity by using sulphuric acid solutions of different specific gravities (Stevens, 1916). Sterilized dishes of uniform size were used for the purpose and a known volume of water and acid-water solutions were put in each to fill about one-fourth of the volume. Petri dishes of uniform size in which equal amounts of the same medium had been poured and inoculated with the fungus,were fixed with gelatine solutions to glass panes big enough to fit on the top of the containers. The lids of the Petri dishes were removed and the glass panes with dishes were sealed with vaseline to the containers. According to this arrangement, the surface of the medium on which the fungus was growing was facing downwards and was exposed to the acid solutions and water exerting known vapour pressures. The linear rate of growth was measured at intervals of three days. The data of the measurement which are the average of two experiments, each running in triplicate, are given in Table I.

TABLE I: LINEAR RATE OF GROWTH OF *C. capsici* AT VARYING ATMOASPHERIC HUMIDITY.

Relative Humidity	Growth in mllimeter		
	3rd day	6th day	9th day
100 percent	9.4	54	65
92 „	12.4	68.8	79.2
78 „	14.6	62.6	77.1
70 „	18.8	57.8	72.7
50 „	22.0	55.4	65.5

From the data presented in Table I it would appear that the best growth occurred at 92 per cent relative humidity. In 100 percent relative humidity, that is in an atmosphere saturated with moisture, the growth was slow. At other humidities, the growth was faster during the first three days but later slowed down.

(iii) *Hydrogen-ion concentration.* The effect of hydrogen-ion concentrations on the growth of the fungus was studied by growing it on modified Richard's solution of Karrer and Webb (1920) to which 2.5 per cent agar was added. Petri dishes of equal size containing equal amounts of media were inoculated in triplicate and the linear rate of growth measured from day to day. The rate of growth noticed after 5 days is presented in Fig. 2 from which it would appear that the optimum hydrogen-ion concentration for the growth of the fungus lies somewhere between 5 and 6.

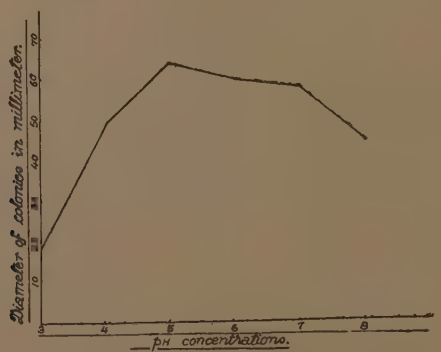

Fig. 2 Growth of *Colletotrichum capsici* in culture in relation to Hydrogen-ion concentrations.

Development of the Disease in Relation to Humidity and Temperature

(i) *Effect of Humidity.* Under field conditions it was observed that the disease usually develops under high humid conditions when rain occurs after the fruits have started to ripen. Park (1930) states that the disease usually breaks out if rainy conditions prevail after the setting of the fruit. Dastur (1921) states that an average relative humidity of over 85 per cent is necessary for the development of the disease. No experiments, however, were conducted by Dastur to confirm his field observations. Experiments were, therefore, conducted to determine the rleation that exists between the prevailing relative humidity and the development of the disease. Twenty-five inoculated ripe fruits were kept in constant humidity chambers for 10 days after which the progress of the disease was measured and the percentage of fruit area destroyed by rot determined. Constant humidities were obtained by use of sodium chloride solutions of different concentrations (Lessage, 1895). A single drop of concentrated spore suspension of *C. capsici* in sterile water was placed at a single selected place on each fruit. The experiment was repeated. The results are presented in Table II.

Table II: Effect of humidity on chilli fruit rot to *C. capsici.*

Humidity	Percentage area of fruits destroyed by rot. Average of 50 fruits at each R.H.
100 per cent	26
95.7 ,,	62
90 ,,	38
84.9 ,,	...
80 ,,	...

From the data presented in Table II it would appear that the maximum disease development occurred at 95.7 per cent relative humidity followed by 90 per cent relative humidity. At 100 per cent relative humidity, in an atmosphere saturated with moisture, the disease development was slow. At 84.9 and 80 per cent relative humidities no infection and disease development took place.

(ii) *Effect of temperature.* The effect of temperature on the development of the disease was studied by placing a number of inoculated fruits in moist chambers kept in incubators maintained at different constant temperatures Each fruit was inoculated at a single central place and the development of the disease was determined by measuring the rotted areas after 10 days and calculating the fruit area destroyed. Twenty five fruits were kept at each temperature and the experiment was repeated. The method of inoculation consisted in placing a drop of concentrated spore suspension of the fungus in sterile water at selected places on each fruit. The effect of temperature on disease development is shown in Table III. It would appear from the data presented in Table III that the greatest disease development took place at 28 °C which is also the optimum temperature for the growth of the fungus in culture.

TABLE III EFFECT OF TEMPERATURE ON CHILLI FRUIT ROT

Temperature.	Percentage area of chilli fruits destroyed by rot. Average of 50 fruits kept at each temperature
15°C	...
25°C	22
28°C	48
32°C	36
40°C	...

PERPETUATION AND DISSEMINATION

A large number of experiments and field trials were conducted to determine the methods of perpetuation and dissemination of the disease. Although in nature the seeds of chillies are infested by the fungus still the disease is not seed-borne and systemic in nature. Studies made revealed that the fungus survives in the fields in plant debris and the primary infection takes place from the spores of the fungus which are wafted by wind from such sources and deposited on the ripe fruits. Secondary infection in the fields takes place from spores which are formed on the fruits first affected. The spores are disseminated mainly by wind, insects and ants visiting the diseased fruits make a very small contribution. By bagging it was possible to raise cent per cent healthy fruits which indicates that the infection takes place from wind-borne spores or from spores carried by insects or ants, from external sources.

CONTROL

As the source of infection is external it was thought that a thorough coverage of the developing fruits by a good fungicide would control the disease. Some spraying trials were, therefore, conducted at Barbheta. After the fruit setting and just before they started ripening, the plants were sprayed with fungicides with a view primarily to cover all the fruits completely and thoroughly with the fungicides. The sprayings were repeated at an interval of 15 to 21 days depending on the weather conditions and in all three to four sprayings were given. The trials were duly replicated and controls kept. Fruits from the treated and control plants were collected and the number of diseased and healthy fruits counted. The results of the spraying trials which were carried out for three years are presented in Table IV.

TABLE IV EFFECT OF SPRAYING ON THE DEVELOPMENT OF CHILLI FRUIT ROT DUE TO *C. capsici*.

Fungicides	1951		1952		1953	
	% sound fruits	% diseased fruits	% sound fruits	% diseased fruits	% sound fruits	% diseased fruits
Perenox[1], 2 lbs. in 100 gals. water	91	9	92	8	95	5
Bordeaux mixture, 2 : 2 : 50	88	12	90	10	92	8
Dithane Z-78[2] 2 lbs. in 100 gals. water.	90	10	88	12	89	11
Yellow cuprocide [1], 2 lbs. in 100 gals, water	92	8	...	...	95	5
Control.	71	29	76	24	72	28

It would appear from the data presented in Table IV that spraying controlled the disease to a great extent and helped production of a higher percentage of sound fruits. All the fungicides tried were almost equally effective. No adverse effect could be noticed from any of the fungicides. It was, however, found that the sprayed fruits stood drying better than the unsprayed fruits. Healthy fruits harvested from the sprayed plots remained healthy after drying and the skin of the dried fruits was bright ruby red. But fruits from unsprayed plots that were healthy when picked, developed diseased spots afterwards. Similar observations were also made by Dastur (1921).

SUMMARY

Studies conducted on the development and control of the ripe fruit rot of chillies due to *C. capsici* have been described. The disease is quite serious and widespread in Assam. From 12 to 32 per cent of the fruits are destroyed. The symptoms of the disease have been described.

In artificial culture it has been found that the optimum temperature for the growth of the fungus is about 28°C. Best growth of the fungus occurs at 92 per cent relative humidity and the optimum hydrogen-ion concentration for growth is between 5 and 6.

Studies carried out showed that under controlled conditions the disease did not develop below 90 per cent relative humidity and the greatest disease development occurred at 28°C.

1. Active Ingredient—Cuprous oxide.
2. Active Ingredient—Zinc ethylenebis (dithiocarbamate).

It was found that the fungus survives in plant debris and primary infection takes place from sources by air-borne spores.

The disease could be controlled by spraying fungicides.

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ANTHRACNOSE OF JUTE

T. Ghosh

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Ikata and Yoshida (1940) described a new anthracnose disease of jute plants occurring in Japan in the Kumamoto Agricultural Station and reported that Tanaka recorded it earlier from Shizuoka Agricultural Station. The pathogen was described by Ikata and Tanaka (1941) as *Colletotrichum corchorum*, a new species. Johnston in 1953 in a private communication* from Kuala Lumpur informed that the *Colletotrichum* isolated from jute in Malaya was tentatively put in the *C. capsici* group.

Hitherto in India and Eastern Pakistan where jute has been extensively cultivated, this disease was practically unknown. Since 1945, however, while working at the Jute Agricultural Laboratories, Dacca, a species of *Colletotrichum* was often found in lesions on stem of jute (*Corchorus capsularis* L.,) alone or in association with *Macrophomina phaseoli* (Maubl.) Ashby, the common parasite of jute. *M. phaseoli* causes extensive damage to both the cultivated species of jute, viz. *Corchorus capsularis* L. and *C. olitorius*. L. through seedling-blight, stem-rot, collar-rot and root-rot. But as the incidence of anthracnose was low and as it was often found in association with *Macro-phomina*, the role of the *Colletotrichum* sp. as a parasite of jute was not very clear till 1950.

In 1950 and 1951 at Chinsurah, West Bengal, epidemic of stem-rot occurred in Japanese-Red, an exotic variety of *C. capsularis;* it was found that the epidemic was due to a species of *Colletotrichum; M. phaseoli* was not found in the affected plants. In 1952, in the variety Japanese-Red and in 3 single plant selections from indigenous types of *C. capsularis*, the disease again appeared in epidemic from at the farm of the Institute at Nilganj. In 1953 the "Broad Leaf Type", a *capsularis* variety evolved from materials obtained from Japan, suffered from a serious epidemic of anthracnose, together with a capsularis hybrid "Deep-three" evolved from indigenous types. In both the cases the pathogen was the same species of *Colletotrichum* which was isolated in 1950 and in subsequent years.

The fungus was isolated in pure culture. Inoculation tests were carried out both with spores obtained from diseased plants and from pure culture on standard potato dextrose agar. The varieties "Japanese-Red", D. 154 and the type "Deep-three" were tried as hosts. While D154, which is the popular cultivated *capsularis* variety was found to be partially resistant, symptoms were evident in about six days in the other two.

Annual recurrence of epidemics in the same varieties, regular isolation of the same pathogen from the infected plants, characteristic anthracnose

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symptoms and the inoculation tests show that the *Colletorichum* sp. is a true parasite of *Corchorus capsularis* L.

The disease starts in the hot and very humid month of July when the crop is about 8 to 10 weeks old. The damage is most severe near about the harvest stage.

The first visible symptom that develops on the green stems is a yellowish-brown moist patch which appears as a depression. The affected spot is irregular in outline, ½ to 1 cm. in length. The number of spots may be one to several. The colour gradually changes to snuff-brown to black, and the depression is more pronounced resulting in necrotic lesions. In some cases one or more of the necrotic spots may increase in size, coalesce and girdle the stem and eventually the stem breaks. This is a characteristic feature of the disease when the attack is severe. In certain varieties of *C. capsularis* the plants may survive up to the flowering stage with cankerous wounds at the nodes or all over the stem. In case of attack on mature plants such cankerous spots may have cracks and the shredding of fibre may result. Sometimes adventitious roots are formed in the region of the cracks. During periderm formation the cankerous wound no longer appears black but changes to deep-brown to snuff-brown at least in the type culture "Deep three". The pods when attacked in young stage turn black and shrivel. Larger pods may survive to produce seed, but black to brown lesions develop on the pods. The seeds are also affected, as a result a large number of affected seeds fail to develop fully. The fungus has been found to be seed-borne. The fibre extracted from infected plants become barky and are consequently classed as "cross-bottom" or "Habijabi" in the market.

The mycelium enters through the epidermis and attacks the parenchymatous tissue between the wedges of bast fibre bundles. The entire parenchymatous tissue of the cortex gradually disintegrates. Under favourable conditions the mycelium attacks the thin walled phloem tissue in-between the fibre bundles. The phloem parenchyma disintegrates and the fibre bundles are exposed. The mycelium often reaches the cambial layer but seldom attacks the wood. Mycelium, however, is seen in the ray cells. During humid days, acervuli appear on the surface of the affected tissue and are visible to the naked eye; under a magnifying lens they appear as bristled hemispherical or slightly lenticular eruptions. The acervuli are organised from subepidermal stroma but are erumpent when mature. The fertile layer is convex in outline. The conidiophores are short, non-septate, simple, straight (25 to 30μ) and hyaline bearing conidia singly. Conidia are acrogenous, falcate, hyaline to sub-hyaline, guttulate 17 – 24 x 2.6 – 3.25μ. Setae deep dark-brown; measure 52 – 138μ, mostly 80μ; base of setae is 3.9μ to 5.2μ, 2–5 septate; a whorl of 10–30 setae are found scattered in each acervulus. Spores germinate, mostly forming appressoria.

The fungus grows very slowly on standard potato dextrose agar. Growth starts with pale greyish-white (sometimes with very pale purplish-tinge) aerial mycelium. The final mycelial growth is appressed, slightly sodden. Innumerable acervuli develop on the surface. Colour of the mycelial matt is masked by formation of abundant acervuli which when

mature are blackish-brown to black. In culture, the acervuli are arranged in concentric zones. In about a week's time at a temperature of 32°C a pale dirty whitish cream coloured mass covers the acervuli. This mass is the conidial accumulation in a semi viscid matrix. In older cultures the colour of the conidial mass changes to warm buff to dirty flesh tint.

The *Colletotrichum* isolated from non-branching *capsularis* types of Japan has been named *C. corchorum* Tanaka & Ikata - a new species. Johnston considered that the *Colletotrichum* isolated from *C. capsularis* in Malaya is allied to *C. capsici*, a culture of which was kindly supplied by him. The type culture of *C. capsici* (Syd.) Butl. and Bisby [GC. 67 I.A.R.I.] was obtained from the Herb. Crypt. Orient, I.A.R.I., New Delhi. A comparative study of the isolates from Malaya and West Bengal showed that they were somewhat allied. The similarity of cultural characters like those of acervuli, setae, conidiophores and conidia of the Malayan and West Bengal strains showed that they were closely allied if not the same. The spore character of GC 67 differed widely from the aforesaid two strains but cultural characters were somewhat similar.

A type culture of the Japanese strain described by Ikata and Tanaka as *C. corchorum* could not be obtained; however, the description of symptoms and of the fungus as given by Ikata and Yoshida (1940) show that they were almost similar to those described in this paper except that the conidia are slightly broader in the Japanese strain. Such variations, however, are possible in the same species: Ikata and Yoshida have observed that conidia of *C. corchorum* Ikata and Tanaka in Kumamoto station were 16.74 x 4.77μ while at Aichi they measured 19.74 x 4.45μ in average. Wide variations have been reported in *C. falcatum* by Rafay (1941, 1953) and Chona (1950). Chona had observed that different strains of *C. falcatum* varied greatly in culture in regard to texture, colour, production of conidia, zonations, stromatoid bodies, etc. Table 1 gives a summary of symptoms and characters of the different pathogens considered to be allied. (see page 66–67).

It appears that the *Colletotrichum* species that attacks jute in Bengal is closely allied to the Malayan strain, both of which are different from *C. capsici* (Syd.) Butl. & Bisby.

It is desirable, therefore, that these two strains, which though slightly different in cultural characters from the strain described by Ikata and Yoshida but having almost similar conidia, be considered as *Colletotrichum corchorum*. Unfortunately, the type culture of *C. corchorum* Ikata and Tanaka could not be obtained. But from the figures and descriptions given in their paper (1940) it appears that the three strains are closely allied and belong to the same species.

Thanks are due to Mr. Ichiro Kato of Tokai-Kinki, Agricultural Experimental Station, Japan, who kindly translated the Japanese paper into English. Thanks are also due to Dr. B. C. Kundu, Director, Jute Agricultural Research Institute, for help and encouragement.

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TABLE I SYMPTOMS AND CHARACTERS OF PATHOGENS CONSIDERED TO BE ALLIED TO *Colletotrichum* SP. UNDER STUDY

	Ikata & Yoshida	Present author		Butler, E.J.
	Anthracnose of *C. capsularis* L.	Anthracnose of *C. capsularis* L.	Anthracnose of *C. capsularis* L.	Anthracnose of *Capsicum annum* L.
	Colletotrichum corchorum Ikata & Tznaka	*Colletotrichum* sp.	*Colletotrichum* sp. from *C. capsularis* from Malaya, reported to be allied to *C. capsici*	*Vermicularia capsici* Syd. = *Colletotrichum capscic* (Syd.) Butl. & Bisby.
Pods	Lesions brown to black.	Lesions brown to black.	Lesions brown to black.	Fruit attacked.
Seeds	Fungus is seed-borne; spores often found on seed coat.	The fungus is seed-bonre; both conidia and setae are found on seed coat.	...	Seeds attacked. ...
Leaves	Lesions are found on leaves.	Lesions are mostly restricted on the midrib and thick veins of the leaf, mostly on under-surface.	Not attacked.	
Stems	Lesions not sunken; definite in outline.	Initially the spots form moist depression in older stems; the lesion no longer form any depression.	Small sunken spots, initially, brown, gradually turn black.	Stem readily attacked.
Acervuli	Black, superficial, scattered.	Black, superficial (erumpent), scattered.	Black, superficial, scattered.	Almost hemispherical.
Stroma	Patelliform, 100–350 μ 25–50 μ high.	Subglobose to hemispherical, sometimes slightly lenticular 130–358 μ.	Hemispherical to lenticular 120–340 μ	Hemispherical 70–120 μ.

TABLE I

	Ikata & Yoshida	Present author		Butler, E.J.
	Anthracnose of *C. capsularis* L.	Anthracnose of *C. capsularis* L.	Anthracnose of *C. capsularis* L.	Anthracnose of *Capsicum annum* L.
	Colletotrichum corchorum Ikata & Tznaka	*Colletotrichum* sp.	*Colletotrichum* sp. from *C. capsularis* from Malaya, reported to be allied to *C. capsici*	*Vermicularia capsici* Syd. = *Colletotrichum capscic* (Syd.) Butl. & Bisby.
Setae	5–15 or several, arising from the margin of the stroma; yellowish brown to deep brown or black. Septa 2–5; 36–117μ long 3.6–5.0μ at the base.	10–30, mostly 20–30 in each acervulum ; deep dark brown; scattered all over the stroma, on margin; septa 2–5; 52–138μ mostly 80μ. 3.9–5.2 at the base.	18–30 in each acervuli, light purplish brown at the tip and darker brown below, septa 2–5. 42–172 μ long and upto 7.0μ broad at the base.	Scattered, dark brown, tips light brown several septa; upto 150μ long.
Condiophore	Simple , hyaline, 15–35μ x 3–4μ bearing conidia singly.	Simple, straight, hyaline bearing conidia singly. 25–30μ.	Simple, hyaline, straight bearing conidia singly 28.0μ – –44μ.	Unbranched, unseptate.
Conidia	Abundant, non-septate, hyaline, curved, bluntly tapered, oblong fusoid to falcate fusoid; 12–25μ x 3.6–6.0μ with 16–22 x 4μ as the most common ones. Germinate forming appresoria.	Acrogenous, falcate hyaline to subhyaline, guttulate; 17–24 x 2.6 –3.25μ, mostly 20μx3.5 Germinate forming appresoria. In mass dirty flesh coloured to warm buff.	Falcate, hyaline, to sybhyaline, guttulate; (18.2–23.8μ) x (4.2–4.9) μ mostly 20.5μ x 4.5μ; in mass dirty flesh coloured to dark warm buff.	In mass pinkish; borne singly at the tip of conidiophore; forms a mass at the base of setae surrounded by mucilagenous matrix. Individually hyaline, unseptate curved narrowed at the ends. 17–28 μ x 3–4μ. Germinate-forming appressoria.

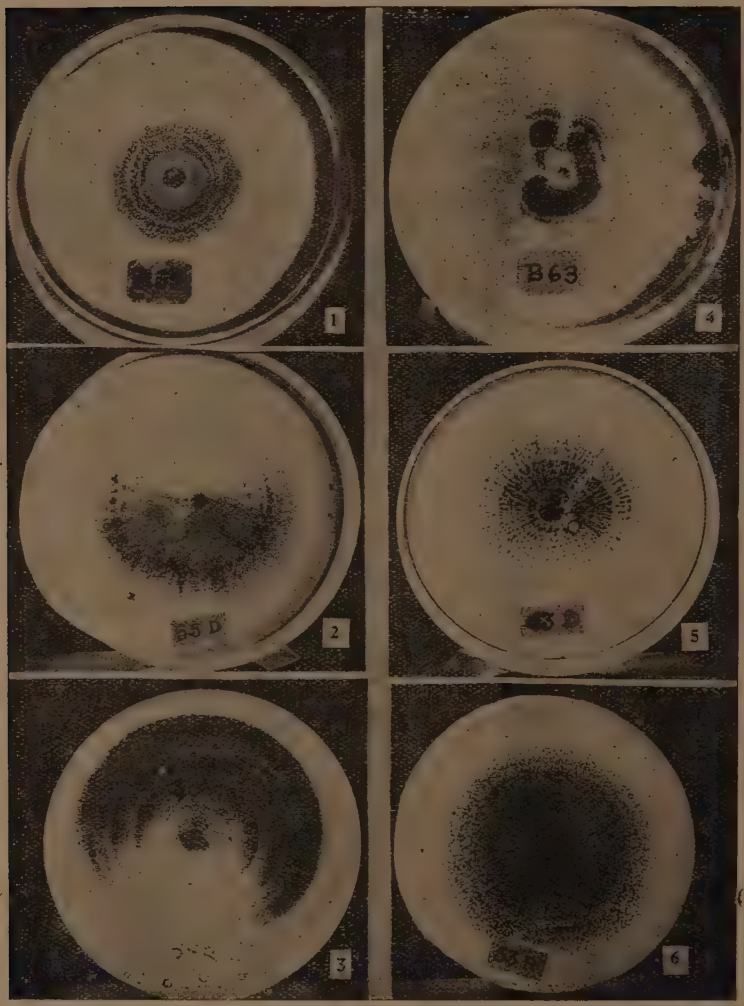

Fig. 1. The *Colletotrichum* sp. isolated from jute in India (hereafter referred to as B55) : twelve days' growth on P.D.A. at 26°C. Humidity below 60 per cent.

Fig. 2. B55 on P.D.A. at 35°C. in continuous darkness for 12 days. Humidity below 60 per cent.

Fig. 3. B55 on P.D.A. at 30°C. under laboratory conditions. Humidity above 80 per cent.

Fig. 4. The *Colletotrichum* sp. from jute in Malaya (hereafter referred to as B63) conditions as for Fig. 1.

Fig. 5. B63 on Jute extract agar. Other condition as in Fig. 2.

Fig. 6. B63 under condition as in Fig. 3

Fig. 1. 45 days' old jute plants (*C. capsularis*) with anthracnose.

Fig. 2. Fibre extracted from mature jute plants having cankers due to anthracnose.

Fig. 3. Conidia of B63 (The Malaya strain) x 550.

Fig. 4. Conidia of B55 (The Indian strain) x 550.

Fig. 5. Conidia of G.C. 67 (*Colletorichum capsici*) x 550.

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WILT DISEASE OF SHISHAM (*DALBERGIA SISSOO* ROXB.) VI. FURTHER STUDIES ON SOIL FUNGI, *ASPERGILLUS* AND *PENICILLIUM*

B. K. Bakshi and Sujan Singh

(Accepted for publication August 6, 1957)

In a preceding number of this series (Bakshi and Sujan Singh, 1956) 19 soil fungi, isolated from soils in shisham forests in the Dehra Dun district, Uttar Pradesh were accounted for. This paper is a continuation of that study and 14 more fungi belonging to *Aspergillus* and *Penicillium* are described.

The methods of study as laid down by Thom and Raper (1945) for Aspergilli and Raper and Thom (1949) for Penicillia were followed in the main. The rates of growth of all fungi were taken in 7 days on malt agar in Petri dishes kept at 25°C in the dark. Cultural characters were also taken on various media under same conditions, unless otherwise stated. The colours were noted from Ridgway (1912). Cultures of all fungi fully described, illustrated and named were sent to Dr. Kenneth B. Raper, who confirmed the identity of some and suggested alternative names for others, which are incorporated. Sincere thanks are due to Dr. Raper for his valued opinion. All the cultures are deposited in the culture collection of the Mycology Branch at the Forest Research Institute, Dehra Dun.

Aspergillus flavipes (Bainier and Sartory) Thom and Church

Colonies fast growing , radius 1.2 cm.; mat on both malt and Czapek's agar solutions appressed felty, white with tinge of yellow ('mustard yellow'), aerial mycelium scanty, the surface studded with numerous conidiophores, the heads of which give a granular appearance to the mat which also appears raised, conidiophores sometimes develop in alternating zones of thick and thin masses, white or with a pale tinge ('tilleul buff'), reverse brown to brownish black; stalks hyaline or light yellow, smooth thick-walled, septate, upto 0.6 mm. long and 8μ broad; conidial heads white, radiate, upto 160μ broad; vesicles hyaline, thick-walled, globose, 20–25μ broad; sterigmata in two series (text-fig. 1a), hyaline, primary 5.7–8.5 x 2.8–5μ, secondary 5.5–7 x 3μ; conidia hyaline or nearly so, globose, smooth (text-fig. 1b), 2.3–2.8μ in diameter.

Identified by Dr. Raper. In *A. flavipes*, the heads are columnar (Thom & Raper, 1945) while they are radiate in the present isolate.

Description of cultural characters. – Le Clerg, 1930; Chaudhuri and Sachar, 1934; Thom and Raper, 1945; Gilman, 1950.

Aspergillus flavus Link

Colonies fast growing, radius 2.5 cms.; mat on malt agar cottony, aerial growth abundant, white when young, green areas representing conidial heads appearing later; on Czepek's solution agar, aerial growth sparse;

stalks light yellow, minutely pitted, 0.4–0.7 mm. long, 8–13μ broad at base, 12–18μ at top; conidial heads light green, radiate, 215 x 100 μ; vesicles hyaline, wall light yellow, globose, 30–47μ broad; sterigmata in 1 or 2 series (text-fig. 2a), former usually in smaller heads and latter in larger ones, also mixed, light yellow, primary 8–21 x 3.5–5.7μ, secondary 4.3–15.5 x 2.8–4.3μ; conidia light yellow, globose to ovoid, wall minutely rough to nearly smooth (text-fig. 2b), 2.8–5.7μ in diameter. Sclerotia white when young, brown on maturity, abundant, round to oval, 185 – 340 x 155 – 340μ.

Identification confirmed by Dr. Raper. Description of cultural characters. – Le Clerg, 1930, 1931; Thom and Raper, 1945; Gilman, 1950.

Aspergillus fumigatus Fresenius

Colonies fast growing, radius 2.9 cms.,: mat on malt agar subfelty becoming felty, white when young, turning green ('slate olive') later; stalks hyaline, wall smooth, 0.1–0.3 mm. long, 3.5–5.5μ broad; conidial heads dark green, columnar 170–285 x 25–42μ; vesicles light green to nearly hyaline, wall light green, globose, 14 – 18 μ broad; sterigmata in one series (text-fig. 3a), light green, 4 – 7 x 1 – 2μ; conidia light green, globose, smooth (text.fig.3b), in solid columns, 2 - 3 μ in diameter.

Identification confirmed by Dr. Raper. Description of cultural characters. – Paine, 1927; Swift, 1929; Chaudhuri and Sachar, 1934; Thom and Raper, 1945; Gilman, 1950.

Aspergillus niger Van Tieghem

Colonies fast growing, radius 2.1 cms., mat on malt agar appressed white containing numerous sclerotia, mycelium scanty reverse unchanged; on Czapek's solution agar subfelty, reverse yellow; stalks yellowish brown, smooth, non-septate, upto 1.5 mm. 10μ wide; conidial heads in shades of purple brown, blackish brown to carbonaceous black. globose, radiate, 115 – 300μ in diameter; vesicles brown, spherical or oval due to flattening at top, thick-walled, 60–120μ broad; sterigmata in two series (text-fig. 4a), brown to brownish black, primary 11–16 x 4.5–7μ, secondary 6–8 x 4–6μ; conidia brownish purple, contents brown, globose, slightly thick-walled, spiny (text-fig. 4b), 2.8 – 4.3μ in diameter. Sclerotia white to pinkish brown with naked eye, light orange in reflected light under microscope. globose, about 0.6–0.7 mm. broad.

Identification confirmed by Dr. Raper, who remarks that the present isolate is an "unusually heavy sclerotium producer".

Description of cultural characters. – Paine, 1927; Swift, 1929; Le Clerg. 1930; Chaudhuri and Sachar, 1934; Thom and Raper, 1945; Gilman, 1950.

Aspergillus quercinus (Bainier) Thom and Church

Colonies fast growing, radius 1.7–2 cms; mat on malt agar appressed with aerial hyphae nearly absent, surface completely and thickly covered with sclerotia, appressed hpyhae hayline, colouration developing over

sclerotia which is white when young soon turning yellow ('naples yellow' to 'mustard yellow') on Czapek's solution agar, pink (Pinkish vinaceous' to 'orange vinaceous') on malt and orange ('cinnamon') on glucose agar, reverse unchanged; yellow and thick-walled, erect, minutely pitted, upto 1 mm. long 9μ broad; conidial heads pale yellow to ochraceous, globose, radiate, upto 85μ broad; vesicles hyaline, thick-walled, globose, 30 – 35μ broad; sterigmata in two series (text-fig. 5a), hyaline, primary 14 – 20x 5 – 7μ, secondary 5.7 – 11.5 x 2.7 – 3.5μ; conidia yellowish, minutely warty, globose (text-fig. 5b), 2.7–3.0μ in diameter. Sclerotia light brown, abundant, 180–300 μ in diameter.

Identified by Dr. Raper. Description of cultural characters. - Le Clerg. 1931; Thom and Raper, 1945: Gilman. 1950.

Aspergillus sydowi Bainier and Sartory

Colonies slow growing, radius 0.9 cm.; mat on malt agar thin, velvety, bluish green , 'slate olive ' on the periphery, reverse unchanged; on Czapek's solution agar wrinkled, 'light calandine green', reverse yellow to red to black with age; growing margin white; stalks hyaline with light green wall, smooth, 0.15 – 0.5mm. long., 4.3 – 7μ broad; conidial heads blue green, globose, radiate upto 115 μ broad; vesicles hyaline with wall light green, globose, usually 8 – 12 μ broad, sometimes extremely reduced; sterigmata in two series (text-fig. 6a), hyaline, primary 5.7 – 13 x 2.1 – 2.8 μ secondary 5.7–8.5 x 2.1–2.8μ; conidia green, spiny, globose (text-fig. 6b), 2.8–4.3μ in diameter.

Identification confirmed by Dr. Raper. Description of cultural characters. – Paine, 1927; Chaudhuri and Sachar, 1934; Thom and Raper, 1945; Gilman, 1950.

Aspergillus ustus (Bainier) Thom and Church

Colonies fast growing, radius 1 – 1.2 cms.; mat on malt agar subfelty, gray, 'light grayish olive', reverse brown; on Czapek's solution agar 'smoke gray to 'mouse gray' deepening to 'chaetura drab'; on glucose agar 'sepia'; conidiophores prostrate on agar, stalks brownish yellow with dark brown thick walls, smooth, 0.005 – 0.18 mm. long and 4.5 – 6 μ broad; conidial heads brown, globose, radiate, upto 60μ broad; vesicles light brown with thick brown wall, slightly elongate, 10 – 16 x 8.5 –12μ; sterigmata in two series (text-fig. 7a) brown, primary 5.7 – 17 x 3μ secondary 4.3 – 8.5x 2.5μ; conidia brownish yellow, globose slightly thick-walled, spiny (Text-fig. 7b) 3.5 – 4.4μ in diameter. Sclerotia absent.

Confirmed by Dr. Raper, who remarks that this isolate is typical. Description of cultural characters. – Thom and Raper 1945; Gilman, 1950.

Aspergillus nidulans (Eidam) Winter

Colonies fast growing, radius 2 cms.; mat on malt agar velvety green ('chromium green'), margin white, reverse unchanged; on Czapek's solution agar velvety with some cottony mycelia over the inoculum, felty with age, green ('deep glaucous green' to 'sage green'), margin white reverse pink; stalks light green, wall greenish yellow, brown on outer portion, smooth,

1. *Aspergillus flavipes.*
2. *Aspergillus flavus.*
3. *Asperigllus fumigatus.*
4. *Aspergillus niger.*
5. *Aspergillus quercinus.*
6. *Aspergillus sydowi.*
7. *Aspergillus ustus.*

49.5 – 112.2 x 3.9 – 5.8μ; conidial heads green with divergent radiating chains of conidia and forming columns in old cultures; vesicles light green, globose, 7.1–11.4μ in diameter; sterigmata in two series (Text-fig. 8a), light green, primary 4.3 – 8.2 x 2.5 – 3.1 μ, secondary 5.7 – 9.9 x 2.1 – 2.8μ; conidia light green, wall deep green, smooth or slightly rough globose, thick-walled (Text-fig. 8b), 3.1 – 5.3μ; perithecia abundant, yellow when young, reddish purple on maturity, surrounded all over by hulle cells (Text-fig. 8f), which are hyaline with thick walls, 13.2– 29.7μ in diameter; asci hyaline when young, rusty red when mature, round to oval (Text-fig. 8c), 9.2 – 11.4μ in diameter; ascospores purple red, lenticular, smooth-walled with two equatorial crests (Text-fig. 8d and 8e), 4.3 – 4.9 x 3.5 – 4.2μ in diameter.

Identification confirmed by Dr. Raper. Description of cultural characters. – Paine, 1927; Chaudhuri and Sachar, 1934; Thom and Raper, 1945; Mohanty, 1948; Gilman, 1950.

Aspergillus unguis (Emil-Weil and Gaudin) Thom and Raper

Colonies fast growing, radius 1.4 cms.; mat on malt agar velvety, green, raised over the inoculum, margin white, reverse unchanged; on Czapek's solution agar smooth, spreading at the margin as irregular lobes, velvety, green ('deep dull yellow green'), reverse unchanged; stalks light brown, smooth, 106 – 192μ long 7.1μ wide below vesicles and 4.3μ wide the lower end; heads green, columnar, 142.0 – 270.0 x 43.0 – 71.0μ; vesicles globose, 9.9 – 12.8μ; sterigmata in two series (Text-fig. 9a), hyaline, primary 5.7 – 8.5 x 2.8 – 4.3μ, secondary 5.7 – 8.7 x 2.8 – 4.3μ; conidia green in mass, greenish yellow under microscope, globose minutely verrucose (Text-fig. 9b) 2.8 – 3.3μ in diameter.

Identification confirmed by Dr. Raper. Description of cultural characters. – Thom and Raper, 1945; Mohanty 1948; Gilman, 1950.

Penicillium lanosum Westling

Colonies fast growing, radius 1.5. cms.; mat on malt gelatin velvety; on Czapek's solution agar consisting of a floccose over-growth arising from a tough mycelial felt, heavily sporing, 'light glaucous blue' to 'pale dull glaucous blue', enclosing a ring of 'light olive gray', to 'tea green',with age turning to 'grayish olive', 'deep grayish olive', to 'dark olive gray', reverse 'avellaneous to 'fawn colour'; penicilli asymmetrically biverticillate, green; conidiophores hyaline, arising directly from the substratum and in some cases as short branches from aerial hypae, rough, 0.1 to 0.35 mm. long, 3.5 – 4.4μ wide; elements in two, sometimes in three series (Text-fig. 10a); metulae 7–12.8 x 2.1–3.1μ; sterigmata 8.5–14.2 x 2.6–2.8μ; conidia yellow globose, minutely rough (Text-fig. 10b), 2.8–4.3μ in diameter; chlamy-dospores in old cultures only, hyaline, terminal and intercalary, 7 – 10μ in diameter.

Identified by Dr. Raper, Description of cultural characters. – Le Clerg, 1931; Raper and Thom, 1949; Gilman, 1950.

Penicillium lilacinum Thom

Colonies fast growing, radius 1.5 cms.; mat on malt gelatin velvety, heavily sporing, 'light grayish vinaceous' turning 'vinaceous drab' over conidial areas; on Czapek's solution agar velvety with some aerial mycelia also, heavily sporing, aerial mycelia 'pale lilac', conidial areas 'light grayish vinaceous', turning soon to 'sorghum brown', reverse of same colour; penicilli asymmeterically biverticillate, metulae are extremely divaricate; conidiophores 'light olive buff', 0.16 – 0.65 mm. long, 2.8–3.5μ broad; elements hyaline, usually in one (Text-fig. 11a), sometimes in two series (Text-fig. 11b), also borne sessile on stalks, acuminate, metulae 10 – 12.8 x 1.80–2.8μ; sterigmata 7.1 – 11.4 x 1.8 – 2.1μ; conidia hyaline, 'pale lilac' in mass globose to elliptic, smooth (Text-fig. 11c), 1.7 – 2.8 μ in diameter.

Identification confirmed by Dr. Raper, who remarks that "This strain is marked by an unusual amount of flocculent aerial hyphae and by conidia (in mass) that are slightly darker than normal for our isolates".

Description of cultural characters. – Le Clerg, 1930; Raper and Thom, 1949; Gilman, 1950.

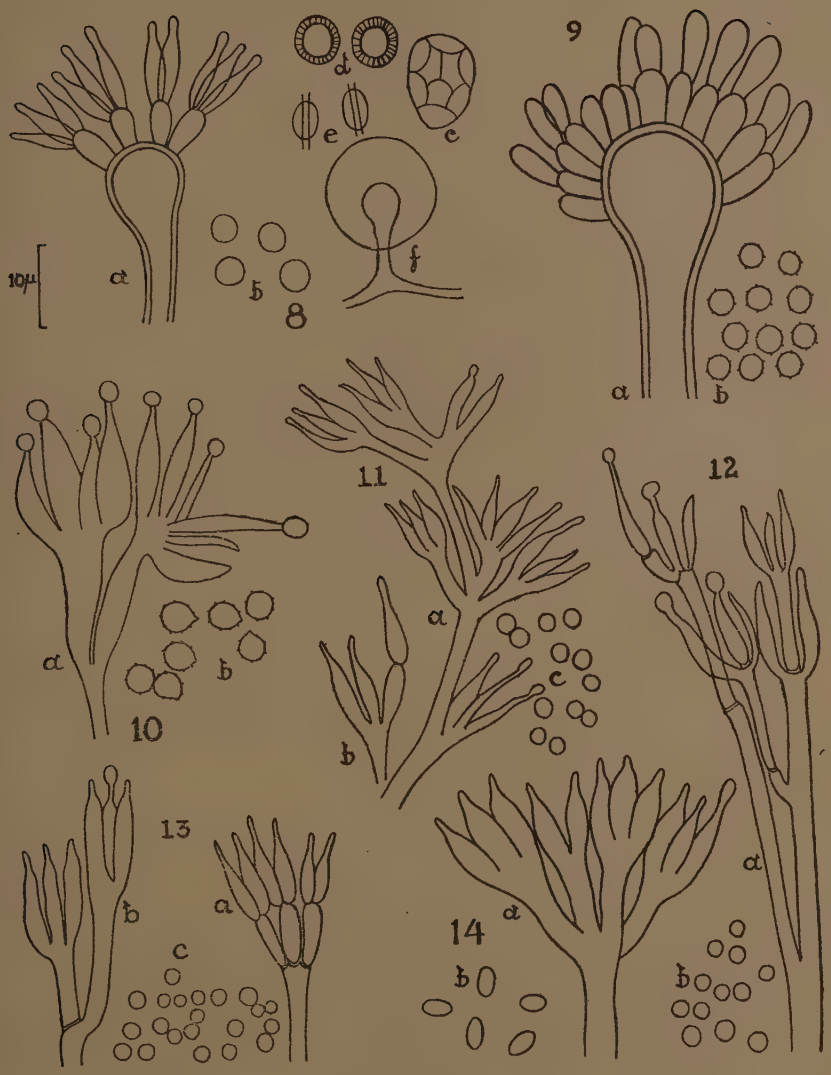
Penicillium asperum (Shear) Rper and Thom

Colonies slow growing, radius 1 cm.; mat on malt gelatin thin floccose; on Czapek's solution agar velvety; at 25°C., sclerotioid perithecia abundant and conidial areas absent, at 18°C., conidial areas predominate without any sclerotioid perithecia and in between 18°C and 25°C., conidial areas and sclerotioid perithecia may both develop sclerotioid perithecial areas 'pale violet plumbeous', turning 'mouse gray' when old, reverse slightly 'vinaceous buff', conidial areas 'greenish glaucous blue' to 'graphalium green', reverse 'pinkish buff ' to 'cinnamon buff' with age; penicilli asymmetrically biverticillate (Text-fig. 12a); conidiophores hyaline, 0.13–0.38 mm. long, 1.6–2.9μ broad; elements hyaline, in two, sometimes in three series; metulae 7.3–16.1 x 1.7–2.9μ; sterigmata 8.8–12.4 x 1.9–2.9μ; conidia light yellow, globose (Text-fig. 12b), 1.6–3μ in diameter. Asci and ascospores not observed.

Identified by Dr. Raper. Description of cultural characters. – Raper and Thom, 1949.

Penicillium funiculosum Thom.

Colonies slow growing, radius 0.6 cm.; mat on malt gelatin 'storm gray' to 'castor gray'; on Czapek's solution agar floccose with rolling hyphae on glass bearing conidia in strands, sporing heavily, conidial areas when young 'courge green' in centre, 'greenish yellow' at periphery, in old cultures 'lettuce green' to 'olive green', drops abundant, brick red, reverse red; Penicilli usually symmetrically biverticillate (Text-fig. 13a), rarely asymmetrically biverticillate (Text-fig. 13b); conidiophores hyaline, 0.1–0.3 mm. long, 2.2–2.9μ broad; elements light yellowish green, in one, two or three series, metulae 10.2–13.1 x 1.7–2.9μ; sterigmata acuminate, 8.8–11.7x 2.2–2.9μ; conidia light yellowish green, round to ovoid, smooth or nearly so (Text-fig. 13c), 2.2–2.9μ in diameter.



8. *Aspergillus nidulans.*
9. *Aspergillus unguis.*
10. *Penicillium lanosum.*
11. *Penicillium lilacinum.*
12. *Penicillium asperum.*
13. *Penicillium funiculosom.*
14. *Penicillium variabile.*

Figures a and b in all cases refer to conidial heads and conidia respectively except figs. 11 and 13 where a and b, conidial heads and c, conidia.

In fig. 8 c, immature ascus; d, face view, e, profile view ascospores; f, hulle cell. All figures x 1300.

Identification confirmed by Dr. Raper. Description of cultural character. – Raper and Thom, 1949; Gilman, 1950.

Penicillium variabile Sopp

Colonies slow growing, radius 0.6 cm.; mat on malt gelatin velvety ('tea green'), reverse yellowish brown ('cinnamon rufous' to 'hazel'), margin white with yellow colour; on Czapek's solution agar velvety, radially 'wrinkled, slightly raised, sporing heavily, green ('dark bluish glaucous' to 'tea gree'), reverse yellowish brown ('cinnamon rufous' to 'hazel'), margin white mixed with yellow coloured mycelia; penicilli symmetrically biverticillate (Text-fig. 14a); conidiophores hyaline, 144–239 x 2.6–2.8 μ; elements hyaline, in two series, metulae 8.5–12.8 x 2.1–4.0μ; sterigimata 11.4–14.2 x 2.1 – 2.8μ; conidia nearly hyaline, bacillar, elliptical to subgobose, apiculate at one end (Text-fig. 14b), 1.7 – 4.4 x 1.4 – 2.8 μ in diameter.

Identified by Dr. Raper. Description of cultural characters. – Raper and Thom, 1949, Gilman, 1950.

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ERRATA to Vol, IX

Page	Line	Read	In place of
74	1	*Puccinia turgida* Syd.	*Puccinia tumidepes* Peck.
„	„	G. G. Dalela	D. D. Dalela
„	2	This is a new record for India.	This is a new host record for *Puccinia tumidepes* Peck.
„	6	*Puccinia turgida* Syd.	*Puccinia tumidepes* Peck.
„	12	„	„

IX INTERNATIONAL BOTANICAL CONGRESS

The Ninth International Botanical Congress will be held in Montreal, Canada, from August 19 to 29, 1959, at McGill University and the University of Montreal. The program will include papers and symposia related to all branches of pure and applied botany. A first circular giving information on program, accommodation, exeursions, and other detail will be available early in 1958. This circular and subsequent circulars including application forms will be sent only to those who write to the Secretary-General asking to be placed on the Congress mailing list:

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Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), p.p. 38-41 and Riker's "The preparation of manuscripts for *Phytopathology*, *Phytopathology* 36 : 953-977, 1946, before preparing their mss. and figures.

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